1967

Determination of seed homogeneity

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DETERMINATION OF SEED HOMOGENEITY

by

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A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Economic Botany

Approved:

Signature was redacted for privacy.

In Charge of Major Work

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Signature was redacted for privacy.

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Iowa State University
Of Science and Technology
Ames, Iowa

1967

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INTRODUCTION

Field crop seed is marketed in lots which range in size from a few pounds to over 100,000 pounds. Large seed lots are usually blends of a number of small seed lots. The blending process may be carried out in several different ways. In many instances, a reasonably satisfactory blend is apparently attained. In other cases, blended lots manifestly lack uniformity.

We do not presently possess a satisfactory method of assaying seed blending. As a consequence, we are unable satisfactorily to evaluate blending methods, blending equipment, or the uniformity of seed lots. A specific example of the marketing ramifications of this problem follows:

> A crop seed lot contains a small percentage of weed and/or other crop seeds (foreign seeds). The foreign seeds are similar in size and shape to the crop seeds. Seed laws require that the concentration of foreign seeds[1] in the lot must be determined and that all seed containers derived from the lot must be labeled identically as to foreign seed content. These containers are shipped to a dozen different locations for retail sale --3 containers to Station A, 16 containers to Station B, -- etc. The lot may be sampled in each location by a government inspector. Each inspection sample will be analyzed; results

[1] Laws also contain other labeling requirements.

of each analysis will be compared with the labeled analysis. If inspection analyses differ from the labeled analysis, a "stop sale" order may be issued on the lot, and the seedsman who was responsible for the labeling will be required to make an adjustment before the seed may be sold. Expense to the seedsman will be twofold: (1) loss of money on this particular lot, and (2) loss of confidence of potential customers who learn of the "stop sale" action.

Most seedsmen are aware of this problem and the fact that no two blending systems are identical. However, the limitations of seed homogeneity tests are such that they have been little used in the past. Tests are needed which can be used, for example, to determine maximum lot size, time required for proper mixing, methods and equipment best adapted to specific jobs, and types of mixtures which may require special attention.

This study is concerned with an evaluation of techniques for measuring seed homogeneity.

REVIEW OF THE LITERATURE

Theoretical Basis for Seed Homogeneity Tests

Foreign seed[1] distribution in crop seed substrates

Leggatt (16) studied the distribution of weed seeds in 98 sacks of timothy which he assumed to constitute a homogeneous lot. One sample was drawn from each bag; one 7-gram sample from each bag sample was analyzed for common weed seeds; and one 14-gram sample from each bag sample was analyzed for noxious weed seeds. Observed frequencies were compared with theoretical Poisson frequencies by means of Chi square tests. Weed seed distribution was in accord with the Poisson distribution.

Leggatt (15) made up bulk lots containing various amounts of stained seeds as follows: red clover containing 1% and 50% stained red clover seeds; alfalfa containing 7%, 10% and 15% stained alfalfa seeds; and bluegrass containing 10% stained bluegrass seeds. From each lot, 1000 100-seed samples, drawn at random, were analyzed for percentage of stained seeds. Theoretical binomial and Poisson distribution curves were calculated. Observed percentages of stained seeds were compared graphically and by means of Chi square tests with the theoretical binomial and Poisson distribution values. Data from the lot containing 1% stained seeds conformed to both the binomial and Poisson distributions. Data from lots containing higher percentages of stained seed followed the binomial, but not the

[1]Weed and/or other crop seeds.

Poisson, distribution.

Leggatt (18) formed the hypothesis that admixture seeds which are smaller than substrate seeds tend to associate in clusters, the mean cluster size being determined by the relative sizes of the seeds of the two species.[1] When this occurs, according to the cluster theory, cluster frequencies rather than individual seed frequencies follow the theoretical Poisson or binomial distribution. Leggatt made a series of tests to test this hypothesis (17). Large bulks of red clover and timothy seeds were mixed with weighed quantities of pigweed and small-seeded false-flax seeds and then were divided into 100-gram samples. Working samples of 14 grams for red clover and 7 grams for timothy were drawn from the 100-gram samples and were analyzed for numbers of weed seeds/present. A total of 472 analyses of red clover and 816 of timothy samples were made. Results in general supported the cluster theory.

Woodbridge (36) found that the numbers of curled dock seeds in 140 1.83-gram samples of orchardgrass drawn from a 256-gram bulk without replacement followed the Poisson distribution. Sixty of the samples were drawn by means of a revolving funnel mixer and 80 were drawn by a pan method. Samp-

[1]Leggatt revised the definition of cluster size after conducting further studies. In a mimeographed booklet which was belatedly published in 1960 (21), effective mean cluster size (c) was defined as the ratio of observed variance to the mean ($s^2/\bar{x}^2$). Leggatt gave mathematical proof of the relationship, $c=s^2/\sigma^2$, and he showed how to determine tolerances for kinds of seeds which exhibit the cluster effect (Appendix F).

ling methods did not alter conclusions concerning distribution of the dock seeds.

In a second study, Woodbridge (37) studied the distribution of seeds of yellow rocket, curled dock, and Canada thistle in a timothy seed bulk. Data from 300 2-gram samples showed that yellow rocket and curled dock were distributed according to the Poisson distribution, but Canada thistle was not. In 50-gram samples, numbers of Canada thistle seeds as well as of curled dock seeds appeared to follow the Poisson distribution. Counts were not made of yellow rocket seeds in 50-gram samples.

Przyborowski and Wilenski (28) studied the distribution of dodder seeds in clover seed lots. They prepared a bag of red clover seed into which 2000 stained dodder seeds were mixed. After bagging, the bag was transported 6 kilometers (bad road) in a four-wheeled wagon. Then, beginning at the top, 500 100-gram samples were taken and examined for dodder content. Dodder seeds were distributed according to the Poisson distribution.

Pzyborowski and Wilenski (28) also discussed data which had been published by Schindler (29). Schindler's data represented results of 5420 analyses for dodder seeds in a red clover seed substrate. Schindler's data, when analyzed by Pzyborowski and Wilenski, were found to conform to the Poisson distribution.

Souleyrette (32), investigated the distribution of vetch seeds in a wheat seed substrate and morning glory seeds in a sorghum seed substrate. Frequencies of occurrence in both instances followed the Poisson distribution.

Shenberger (30) mixed one Johnsongrass seed, two Canada thistle seeds, four perennial sowthistle seeds, six field peppergrass seeds and nine giant foxtail seeds into 150 grams of red clover. A 50-gram sample was taken from the 150-gram bulk, analyzed for number of weed seeds present, and returned to the bulk. The process was repeated until 48 samples had been analyzed. Chi square tests, made to compare observed frequencies with expected frequencies, revealed no significant deviations from the Poisson distribution.

Data which appear to contradict the hypothesis that low concentrations of weed seeds become distributed in a crop seed bulk according to the Poisson distribution were reported by Dufrenoy, Dusseau and Renier (6). These workers studied the distribution of seeds of Trifolium (species not stated) in 197 5-gram samples of alfalfa from a commercial seed lot. The mean number of Trifolium seeds per sample was 3.72. More 5-gram samples contained zero and one seeds of Trifolium than would be expected in a Poisson distribution. The authors were able to fit the data to a Pearson curve type J_1.

Related studies

Fisher, Thornton, and MacKenzie, in 1922 (7), derived the index of dispersion,

$$\chi^2 = \frac{\Sigma(x-\bar{x})^2}{\bar{x}} \quad ,$$

for use in comparing numbers of bacterial colonies in soil samples. These workers applied the index of dispersion to several hundred sets of bacterial counts, each set being comprised of the counts from three, four, five, six or nine plates. They concluded that under ideal conditions (i.e., when there are no nutrient deficiencies in the test medium, no organisms present which affect bacterial reproduction, etc.), bacterial counts on replicate plates will vary in the same manner as samples from a Poisson series; when these conditions are fulfilled, the mean bacterial count of a number of plates is a direct estimate of the population mean; and any significant departure from the theoretical Poisson distribution is a sign that the sample mean is an unreliable estimate of the population mean.

Sukhatme (33) conducted experiments to determine the lower limit of the mean, above which the index of dispersion supplies a satisfactory test of significance for homogeneity. Eight hundred samples of five observations were drawn from tables of random numbers of each of the five Poisson populations having means of one, two, three, four, and five. Successive pairs of

samples of five observations were combined to make samples of 10 observations, and successive groups of three samples (each sample composed of five observations) were combined to make samples of 15 observations. Indices of dispersion (χ^2 values) were then calculated for samples of 5, 10, and 15 observations. The calculated Chi square values were grouped in frequency distributions and compared with the theoretical distribution of Chi square. Sukhatme concluded that the index of dispersion measures homogeneity in a Poisson series provided that the mean is greater than one.

Morgan, MacLeod, Anderson, and Bliss (26) studied the distribution of bacterial clumps in microscopic fields as a preparatory step in developing an improved technique for grading milk. Counts agreed moderately well with the random expectation of a Poisson series within a range of 0.18 to 1.05 bacterial clumps per field. This information was used for developing sequential inspection plans.

Tests for Seed Homogeneity

Available tests

A test for determining homogeneity in seed was described by Leggatt in 1951 (19) and was made a part of the 1953, 1956, and 1959 editions of the International rules for seed testing (10, pp. 43-48; 11, pp. 44-49; 12, pp. 556-561). This test, the Leggatt homogeneity test, provided instructions for deter-

mining homogeneity with respect to numbers of weed and/or crop seeds (foreign seeds) in a unit weight, purity percentage, and germination percentage. The test is a Chi square test which measures the dispersion of observed values around the mean. The statistic employed, the Figure of Homogeneity, is the statistic (the index of dispersion) that was derived by Fisher, Thornton, and MacKenzie in 1922 (7). Recommendations were not made by Leggatt concerning the number of bags which should be sampled or size of samples which should be examined. A condensation of the Leggatt homogeneity test is included in this thesis (Appendix A).

In 1960, Miles, Carter, and Shenberger proposed a Long and a Short test for determining homogeneity (25). The Long homogeneity test (Appendix B) consists of making an F test by dividing sample variance[1] by the "maximum variance permitted for a homogeneous lot". The computed F value is then compared with tabulated F values. The value for the "maximum variance permitted in a homogeneous lot" is obtained by multiplying expected variance by a factor (1.69 for nonchaffy seeds and 3.24 for chaffy seeds).

The Short homogeneity test (Appendix C) consists of analyzing samples from individual bags of a lot and comparing the range of counts or percentages obtained with tabular values of

[1]Variance is a numerical measure of the degree of dispersion of individual values about the mean.

the "maximum range for homogeneity".

Westmacott and Linehan (35), working with seed purity only, suggested that the Leggatt homogeneity test -- which draws a hard, fast line between homogeneous and heterogeneous lots -- should be replaced with a statistic which measures extent of heterogeneity. The statistic that they proposed was defined as

$$h = \frac{\text{Observed variance}}{\text{Theoretical minimum variance}} .$$

They proposed that limits of acceptability be designated subsequent to the accumulation of data which would indicate h values that could reasonably be achieved with conventional seed mixing procedures. They suggested that these acceptability limits, if possible, should be the same for different sizes of seed lots and for different quality grades of seed.

In 1962, Miles (24) recommended use of the statistic,

$$H = \frac{\text{Observed variance}}{\text{Theoretical minimum variance}} - 1 .$$

Miles believed that the critical H value, the value beyond which lots would be considered heterogeneous, could be determined subjectively and from experience with commercial seed lots only. Miles stated: "A critical H value should be determined only from H values obtained from lots selected at random, or from all lots encountered over a considerable time period. H values from lots selected for heterogeneity test because the lots were suspected to be heterogeneous should not

be used in determining a critical H value." Miles recommended that constant sample sizes be used for determining H values: 100 seeds for germination, approximately 1000 seeds for purity, and approximately 10,000 seeds for weed seed numbers.

The homogeneity test outlined by Miles (24), the H homogeneity test, was included in the 1966 International rules for seed testing (13, pp. 140-144) as a replacement for the Leggatt homogeneity test which had been in earlier editions. Instructions for the H test specify the number of bags which are to be sampled (which varies with the size of the lot), the minimum size of each bag sample (about 12,500 seeds), and the minimum size of each working sample (about 10,000 seeds). The basis for these recommendations is not recorded in the literature. The H homogeneity test is summarized in Appendix D of this thesis.

Effectiveness of available tests

Experience in the use of the above enumerated tests has been limited. Pertinent reports follow.

According to Miles, Carter, and Shenberger (25), the test in the 1956 International rules for seed testing (the Leggatt homogeneity test) is unrealistic. These authors state:

> "[The International rules for seed testing in prescribing the Leggatt homogeneity test] assume perfect mixing of seeds; this is unattainable. In addition, they make no allowance for within-bag segregation; they assume that individual-bag samples are reduced to working samples truly at random, that is, by the best mechanical equipment; and they assume that the work of analysts is per-

> fect. In other words, the Rules allow for random sampling variation only. Moreover, the tests require an unnecessary amount of computation."

Westmacott and Linehan (35) applied the Leggatt homogeneity test to pure seed percentages of samples from eight large seed bulks of Lolium perenne known to have been mixed according to normal commercial practice; only one bulk of seed satisfied the test for homogeneity. The authors state: "It is probable that if the working samples were large enough, no lot of seed would ever be declared homogeneous on the basis of the ISTA test (the Leggatt homogeneity test). Clearly at the present the best method of getting a lot passed as homogeneous by the present ISTA test is to take few and small samples."

The Leggatt homogeneity test was used by Parkman (27) for evaluating the performance of batch and continuous flow seed blenders and by Kent (14) for measuring homogeneity of 18 seed lots sampled at 12 commercial seed processing plants. Neither of these authors expressed concern over the validity of usefulness of the test.

There have been no published commentaries concerning the Long or Short homogeneity tests of Miles, Carter, and Shenberger (25).

Westmacott and Linehan (35) used the h statistic to test 458 lots of Lolium perenne and 247 lots of L. multiflorum for homogeneity with respect to pure seed percentage. Among the L.

perenne lots, a critical h value of 3.00 would have been required for 75% of the lots to be considered uniform. For L. multiflorum, an h value of 4.00 would have been required for 72% of the lots to pass as being sufficiently homogeneous.

Linehan and Mathews (22) used the H statistic for testing the uniformity of 816 lots of Lolium perenne with respect to pure seed percentage and number of weed seeds. They also tested 349 lots of L. multiflorum for homogeneity with respect to pure seed percentage, number of weed seeds, and percentage of awned seeds. Samples were drawn from five bags of each lot. Working samples of 5 grams (about 2500 seeds) from each bag sample were tested. Critical H values of 1.00 and 2.00 would have allowed over 75% of the L. perenne lots to be adjudged as sufficiently uniform with respect to pure seed percentage and number of weed seeds, respectively. In L. multiflorum lots, H values under 1.00 for pure seed percentage were obtained for only 63.6% of the lots, and H values under 2.00 for number of weed seeds occurred in only 39.6% of the lots. H values of over 5.00 for awned seed percentage were observed in 37.2% of the L. multiflorum lots. Significant correlations were observed for both species between H values for number of weed seeds and pure seed percentage.

Related studies

Danckwerts (5), in a discussion of mixing theory, pointed out that any mixture, if scrutinized closely enough, will show

regions of segregation; the decision of whether or not a mixture is well mixed is dependent upon the purpose for which the mixture is intended.

Cochran (in estimating concentration of wire worms in field plots; Poisson distribution assumed) found that much more information was obtained by doubling the number of samples taken than by doubling the size of samples. The procedure used in analyzing the data is explained in detail in Cochran's paper (3).

When numbers being analyzed follow the Poisson distribution, data should be transformed before an analysis of variance is made (31, p. 314). The square root transformation is usually sufficient, but yields only approximations. A more exact procedure has been described by Cochran (3).

Matches (23) provided a detailed outline of the procedure he used for determining optimum mower strip size and optimum number of sample units per mower strip for comparing yields of different types of pastures. Matches' experiment differed from most uniformity trials since he sampled at random from only a portion of the fields under study.

Tolerance Tables

Tolerances for rates of occurrence of noxious weed seeds are used routinely in seed law enforcement work. A copy of the tolerance table used in administration of the Federal Seed

Act (34) and instructions for using the table are reprinted in Appendix E of this thesis.

MATERIALS AND METHODS

General

Batches of seed were mixed to different degrees of uniformity. Batch uniformity was estimated by calculating the variance of numbers of indicator seeds[1] present in samples from the batch. The effectiveness of seed homogeneity tests for evaluating degree of uniformity of batches mixed to different degrees was then determined.

Data were processed in the Iowa State University Computation Center.

Experiment 1

Five kinds of indicator seeds were mixed into a substrate of pure unstained alfalfa (Medicago sativa) seed. Indicator kinds were: red-stained alfalfa seeds, blue-stained alfalfa seeds, curled dock (Rumex crispus) seeds, Wild mustard (Brassica kaber) seeds, and prostrate pigweed (Amaranthus graecizans) seeds. The red- and blue-stained alfalfa seeds were assumed to differ from substrate seeds in color only. (This premise was experimentally validated). Since each kind of indicator seed was distinctly different in appearance from each other kind as well as from seeds of the substrate, analytical errors (hopefully) were absent.

[1] An indicator seed is one differing sufficiently from substrate seeds to allow easy detection.

Each indicator seed kind was present in an approximate concentration of 1:100, indicator:substrate. Weights per 100 seeds of the species used were: alfalfa, .219 grams; curled dock, .119 grams; wild mustard, .222 grams, and prostrate pigweed, .098 grams. Total weight of each batch was 160 grams.

The mixing apparatus consisted of an Erlenmeyer flask and the top portion of a Boerner seed sampler to which a funnel was mounted. Thus, the apparatus possessed a pouring spout. The mixing procedure is described in Figure 1.

The number of indicator seeds of each kind in each 2-gram sample was recorded. Variances were calculated for numbers of indicator seeds present in 2-, 4-, 8-, and 16-gram samples. Data for 4-gram samples were obtained by combining data for each consecutive pair of 2-gram samples. Data for 8- and 16-gram samples were obtained by combining data for each consecutive set of four and of eight 2-gram samples, respectively (Table 15).

Batches were tested for homogeneity with respect to each kind of indicator seed by means of the Leggatt homogeneity test (Appendix A), the Long homogeneity test (Appendix B), the Short homogeneity test (Appendix C), and the H homogeneity test (Appendix D).

The numbers of seeds of each kind of indicator seed in each sample of each batch were compared with entries in the Federal Seed Act tolerance table (Appendix E). The percentage

Figure 1. Mixing procedure used in Experiment 1

1. Substrate seeds were placed and leveled in the top of the mixing apparatus while the spout was closed; seeds of each kind of indicator were placed in predetermined positions on top of the substrate (a).

2. The spout was opened so that the seeds could flow into the flask (b). When all seeds in the batch had emptied into the flask and had been returned to the top of the apparatus (spout closed; c), the seed was said to have been mixed one time. Batches of varying degrees of uniformity were prepared by mixing 2, 3, 4, 8, or 16 times.

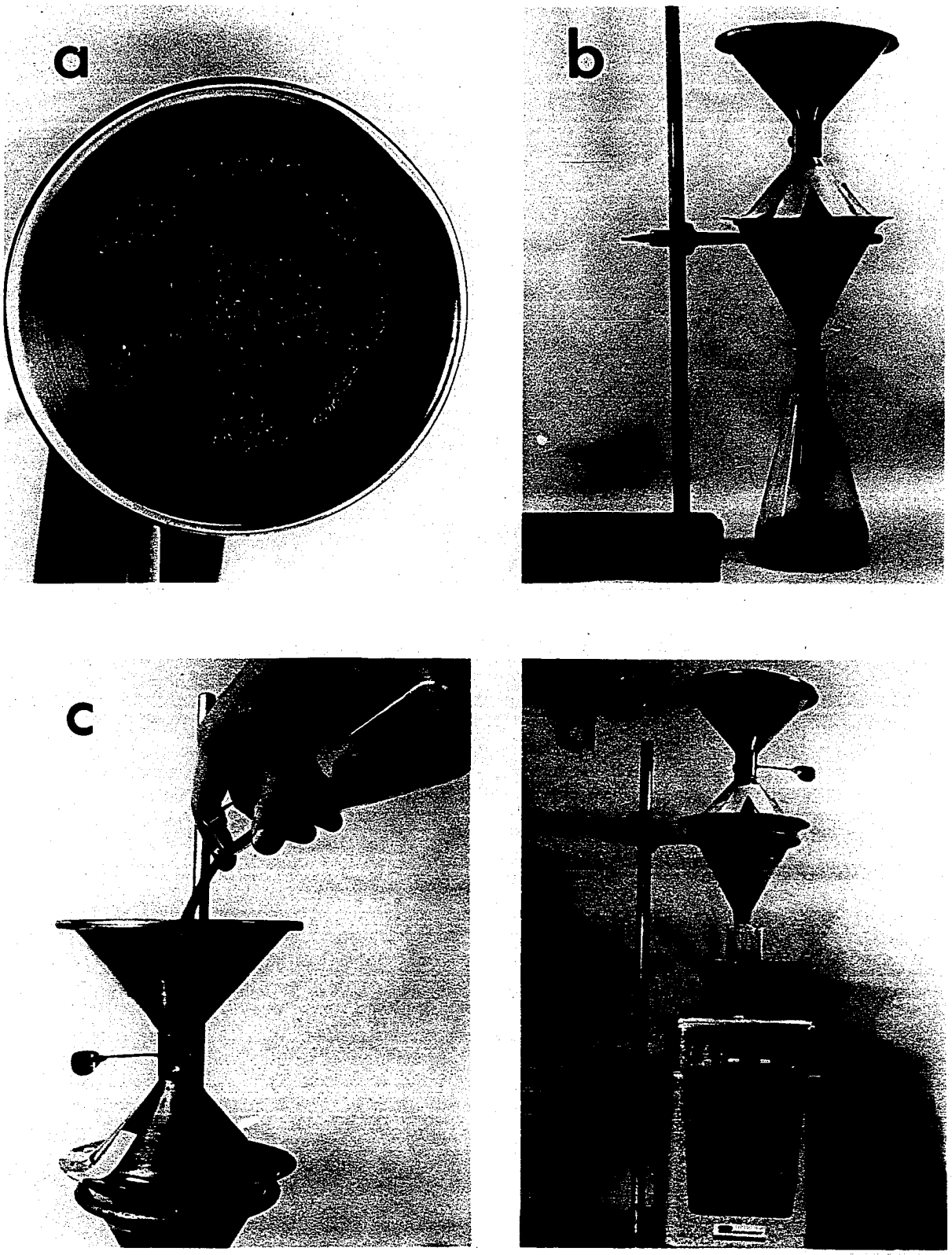
3. After a batch had been mixed the desired number of times, 2-gram samples were allowed to flow into a 50-ml. beaker on a direct-reading analytical balance (d). Seed flow was controlled by opening and closing the spout. Sample weight seldom deviated by more than 0.10 of a gram from the desired 2.00 grams. Samples were numbered consecutively, 1 to 80, as taken from the mixing apparatus.

   a. Positioning of indicator seeds on top of substrate in top portion of mixing apparatus; indicator seeds (reading clockwise) were: red-stained alfalfa, prostrate pigweed, curled dock, wild mustard, and blue-stained alfalfa.

   b. Spout open; seed flowing into flask.

   c. Spout closed; seed returned to top of mixing apparatus.

   d. Position of mixing apparatus in relation to direct-reading balance.


a
b
c

of samples containing indicator seeds in numbers which exceed Federal tolerance limits was determined for each mixing treatment-indicator seed kind-sample size combination.

Experiment 2

Mixing pattern of a single kind of indicator seed was determined in five different substrates of rape seed (Brassica napus). These substrates were derived from a single commercial seed lot.

Determination of specific gravity and size of substrate seeds

Bulk specific gravity determinations were made with an air comparison pycnometer, Beckman Model 930. This apparatus measures the volume of air displaced by a weighed quantity of seed.

Determinations of specific gravities of individual seeds were made in solutions of cupric chloride. Information concerning solute concentrations which would provide desired solution specific gravities was obtained in the Handbook of chemistry and physics (8, p. 1997). Solution concentrations were adjusted, if necessary, after the weight of a known volume of each solution had been determined. One hundred seeds were tested at a time. The seeds were placed in the solution having the heaviest specific gravity. Seeds were stirred to eliminate surface tension effects. All seeds which floated were removed from the solution and were placed on blotting paper

for removal of excess solution. These seeds were then placed in the solution having the second-heaviest specific gravity and stirred. Floating seeds were removed, blotted, and placed into the next solution in the series. The process was repeated through the remaining solutions. The number of seeds in each specific gravity class was recorded.

Seeds were screened into the following seed size classes:

Seed size class	Sieve size
Large	Over 1/12" round-hole sieve
Medium large	Through 1/12" sieve; over 4/64" x 3/4" sieve
Medium	Through 4/64" x 3/4" sieve; over 10 x 10 mesh sieve
Medium small	Through 10 x 10 mesh sieve; over 1.651 mm. round-hole sieve
Small	Through 1.651 mm. sieve

Substrates

Five substrates were compared:

Substrate 1. Ungraded seed from the original rape seed lot; black (natural color).

Substrate 2. Medium size; ungraded for specific gravity; black.

Substrate 3. Medium size; ungraded for specific gravity; yellow (bleached).

Substrate 4. Ungraded for size; medium specific gravity (1.025 to 1.075, graded in cupric chloride solutions); black.

Substrate 5. Medium size; medium specific gravity; black.

Indicator seeds

Indicator seeds used in Substrates 1, 2, 4, and 5 were bleached in sodium hypochlorite and stained with Auramine 0 to insure their immediate detection in samples being analyzed. Indicator seeds used in Substrate 3 were unstained. The specific gravity of all indicator seeds was between 1.025 and 1.075, as determined in cupric chloride solutions. All indicator seeds passed through a 4/64" sieve but remained on top of a 10 x 10 mesh sieve.

Procedure

Each batch weighed 320 grams. This provided approximately the same number of seeds per batch (73,000) as were tested in each batch in Experiment 1. An approximate 1 : 100 ratio of indicator : substrate was used. Seeds were mixed by pouring the seed through the mixing apparatus 3 times. Twenty 16-gram samples were drawn from each batch.

Variances were calculated for numbers of indicator seeds in samples of each batch. In addition, data from all replications of each treatment were pooled to provide better estimates of the variation occurring within each substrate.

Experiment 3

Numbers of blue-stained alfalfa seeds occurring in 2-, 4-, 8-, and 16-gram samples in Experiment 1 were randomly assembled into groups of 5, 10, and 20. A table of random

numbers (31, pp. 10-13) was used as an aid in selecting individual values from pooled raw data of 4 replications of batches that had been mixed 2, 4, or 16 times. There were 10 replications of random samples for each sample size-group size-mixing treatment combination.

Variances were calculated for each group, and each group was tested for homogeneity by application of the Leggatt homogeneity test (Appendix A), the Long homogeneity test (Appendix B), the Short homogeneity test (Appendix C) and the H homogeneity test (Appendix D).

The variance of the mean was calculated from the pooled data of 10 replications for each group size-sample size-mixing treatment combination.

Experiment 4

One indicator seed kind (rape seed, medium size[1], specific gravity between 1.025 and 1.075, bleached and stained yellow) and one substrate (rape seed, ungraded for size or specific gravity, black color) were used in Experiment 4. Data were obtained from various combinations of mixing treatment, indicator seed concentration, and sample size. Each 320-gram batch was sampled in its entirety. Numbers of samples per batch were 80, 40, 20, and 10 when sample sizes were 4, 8, 16, and 32 grams, respectively. Each treatment combination was replicated

[1]Through 4/64" x 3/4" sieve; over 10 x 10 mesh sieve.

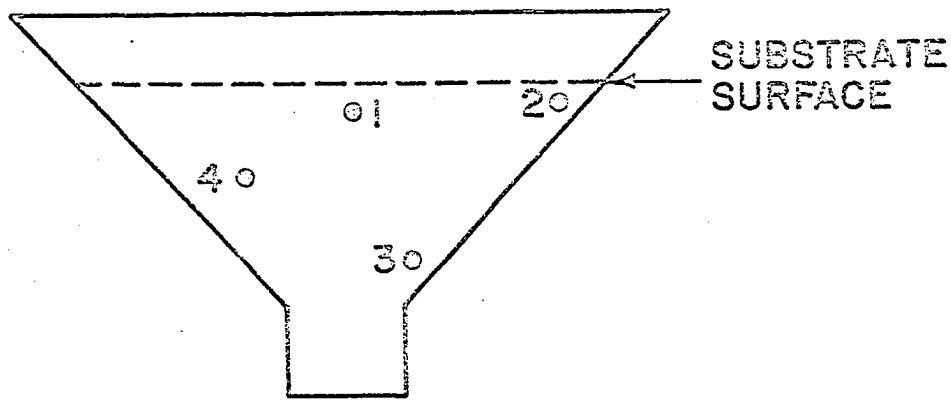
four times.

Variances were determined for numbers of indicator seeds in samples from each batch. Each batch was tested for homogeneity by application of four seed homogeneity tests. The percentage of samples containing indicator seeds in numbers which exceed Federal tolerance limits (Appendix E) was determined for each treatment combination.

Experiment 5

The purpose of Experiment 5 was to determine the effect of starting position of indicator seeds in the mixing apparatus upon variance of indicator seed counts after mixing. Unstained alfalfa seed (160 grams per batch) was used as the substrate; red- and blue-stained alfalfa seeds (400 seeds of each kind per batch) served as indicator seeds.

Four tests were made. In test 1, red-stained alfalfa seeds were placed in Position 1 and blue-stained alfalfa seeds were placed in Position 2 prior to mixing (see diagram).



Batches were mixed by pouring through the mixing apparatus 2 times. Placement of indicator seeds for Test 2 was identical to that of Test 1, but batches were mixed 3 times instead of 2 times. Test 3 consisted of mixing batches 2 times following the placement of the red-stained seeds in Position 3 and the blue-stained seeds in Position 4. Test 4 differed from Test 3 only in times of mixing -- 3 times instead of 2. Each test was replicated 4 times.

Twenty 8-gram samples were withdrawn from each batch. Indicator seeds of each kind in each sample were counted. Within-batch variances of numbers of indicator seeds were calculated for each position-time of mixing combination. Data were analyzed by a standard analysis of variance.

RESULTS

Experiment 1

Variance, a statistic often used for estimating the degree of dispersion in experimental data, was calculated for numbers of each kind of indicator seed in each sample size in each batch tested. In addition, a pooled variance was calculated for each indicator seed kind-sample size-mixing treatment combination. Variances and means have been tabulated in Tables 16 through 19.

Pooled variance of numbers of indicator seeds in 2-gram samples was plotted against mixing time for the five indicator seed kinds (Figure 2). In general, there were lower variance values of numbers of indicator seeds in samples from batches mixed 3 times than from batches mixed 2 times; slightly lower variance values from batches mixed 4 times than from those mixed 3 times; but no additional effects on variance for mixing times beyond 4. The mixing patterns for red- and blue-stained alfalfa seeds were similar, but each of the other kinds of indicator seeds behaved differently.

Partial explanation of the differences in variance among the different kinds of indicator seeds is given in Table 1. Whereas red- and blue-stained alfalfa seeds were randomly distributed among the different samples after 8 or 16 times of mixing, seeds of curled dock and wild mustard occurred with highest frequency in the last-drawn samples. Prostrate pigweed

seeds also occurred in greater numbers in the last-drawn samples, but to a lesser extent than curled dock and wild mustard seeds. Variance of numbers of curled dock and wild mustard seeds was greatly reduced when counts from the last-drawn samples were omitted from the analysis (Table 2).

Table 1. Experiment 1. Number of seeds of five kinds of indicator seeds in successively-drawn 16-gram samples from 160-gram batches; unstained alfalfa seed substrate; average of four replications[a]

Indicator seed kind	number of times mixed	Sample number			
		1 through 8		9	10
		Range	Average	Average	Average
Red-stained alfalfa	8	65-79	73	67	75
	16	67-78	74	66	74
Blue-stained alfalfa	8	64-85	75	66	73
	16	69-84	74	73	74
Curled dock	8	63-80	71	80	108
	16	67-77	72	79	103
Wild mustard	8	60-83	68	76	121
	16	59-84	71	85	124
Prostrate pigweed	8	63-79	72	75	78
	16	62-78	70	64	89

[a]Samples were numbered consecutively as they were taken from the mixing device.

Results obtained from the Leggatt homogeneity test (Appendix A), the Long and Short homogeneity tests (Appendix B, Appendix C), and the H homogeneity test (Appendix D), applied

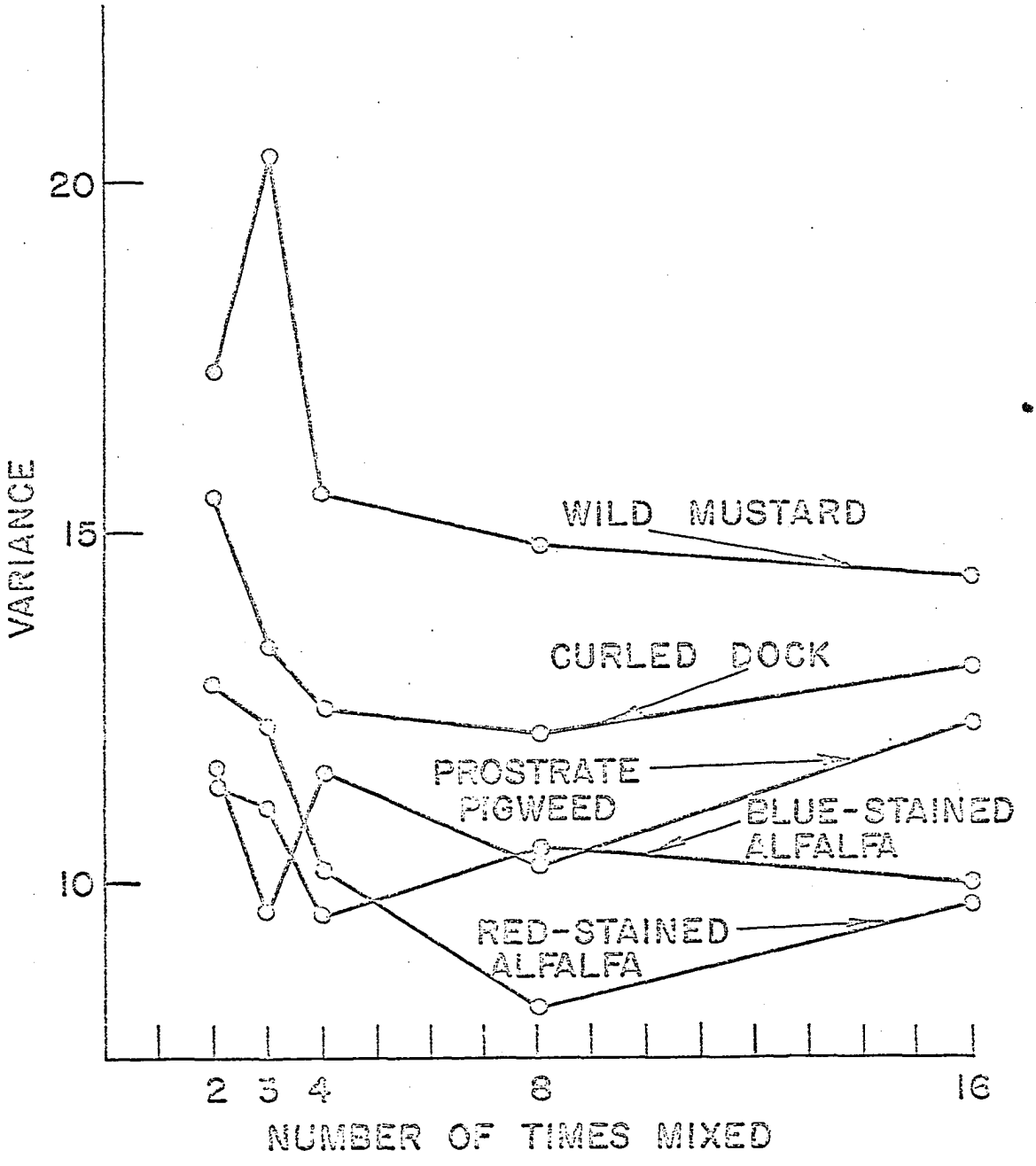


Figure 2. Experiment 1. Variance of numbers of five kinds of indicator seeds in an unstained alfalfa seed substrate; 2-gram samples; average of approximately 9.5 indicator seeds per sample

Table 2. Experiment 1. Comparison of variances of numbers of five kinds of indicator seeds in 8, 9, and 10 16-gram samples. Average of four replications[a]

Indicator seed kind	Number of times mixed	Number of samples included in analysis		
		8[b]	9[c]	10[d]
Red-stained alfalfa	8	72.60	70.53	83.61
	16	54.13	68.88	62.69
Blue-stained alfalfa	8	77.43	81.35	81.07
	16	80.28	70.73	64.43
Curled dock	8	150.45	150.80	273.61
	16	102.65	97.08	198.66
Wild mustard	8	92.05	89.85	366.87
	16	85.55	106.05	419.50
Prostrate pigweed	8	223.90	200.18	195.07
	16	131.20	129.60	175.38

[a]Samples were numbered consecutively as they were taken from the mixing device.

[b]Samples No. 9 and No. 10 omitted from analysis.

[c]Sample No. 10 omitted from analysis.

[d]All data included in analysis.

to the data of Experiment 1, are summarized in Table 3. More samples were declared heterogeneous when the Leggatt homogeneity test was applied than when the other tests were employed. The H homogeneity test was the second most severe test. The least severe tests, the Long and Short homogeneity tests, provided similar evaluations as indicated by total lots

Table 3. Experiment 1. Comparison of homogeneity tests. Results combined for five mixing treatments and five indicator seed kinds[a]. Data presented in terms of heterogeneity declarations (100 possible)

Sample size (grams)	Test applied			
	Leggatt	H	Long	Short
2	44	5	4	Test not made[b]
4	51	18	11	
8	58	45	25	27
16	53	51	29	30
Total for all sample sizes (400 possible)	206	119	69	-

[a]Data for individual indicator seed kinds and for individual mixing treatments are given in Tables 20 through 24.

[b]Limits for tests involving over 20 observations were not given by the authors (25).

declared heterogeneous, but these two tests sometimes differed in evaluation of specific lots (Tables 20 through 24).

All of the tests had at least limited capacity for distinguishing between samples containing stained alfalfa seeds that had been mixed to differing degrees of uniformity (Tables 20, 21). Tests for distinguishing among amounts of mixing by measuring homogeneity with respect to the three weed seed kinds are difficult to interpret because of the peculiar distribution patterns of the weed seeds.

All four homogeneity tests were more severe when tests were made of large samples than of small samples.

Percentages of samples which contained numbers of indicator seeds in excess of tolerance limits of the Federal Seed Act (Appendix E) are recorded in Table 25. The percentage of samples containing indicator seeds in excess of tolerance was related to the number of times that the seed had been mixed (Figure 3). In poorly mixed seed (mixed 2 times), a greater percentage of indicator seed counts was outside of tolerance in large samples than in small samples. In well-mixed seed (mixed 16 times), few samples of any size contained excess numbers of indicator seeds.

Experiment 2

Size, weight, and average specific gravity of rape seeds are listed in Table 4. The percentages of seeds in different specific gravity classes were different for each size class (Figure 4).

Specific gravity values determined by use of the air comparison pycnometer were consistently higher than average values determined in cupric chloride solutions (Figure 4).

Differences in substrate composition did not affect variance of numbers of indicator seeds in 16-gram rape samples (Table 5). Three analytical procedures were used for evaluating the data:

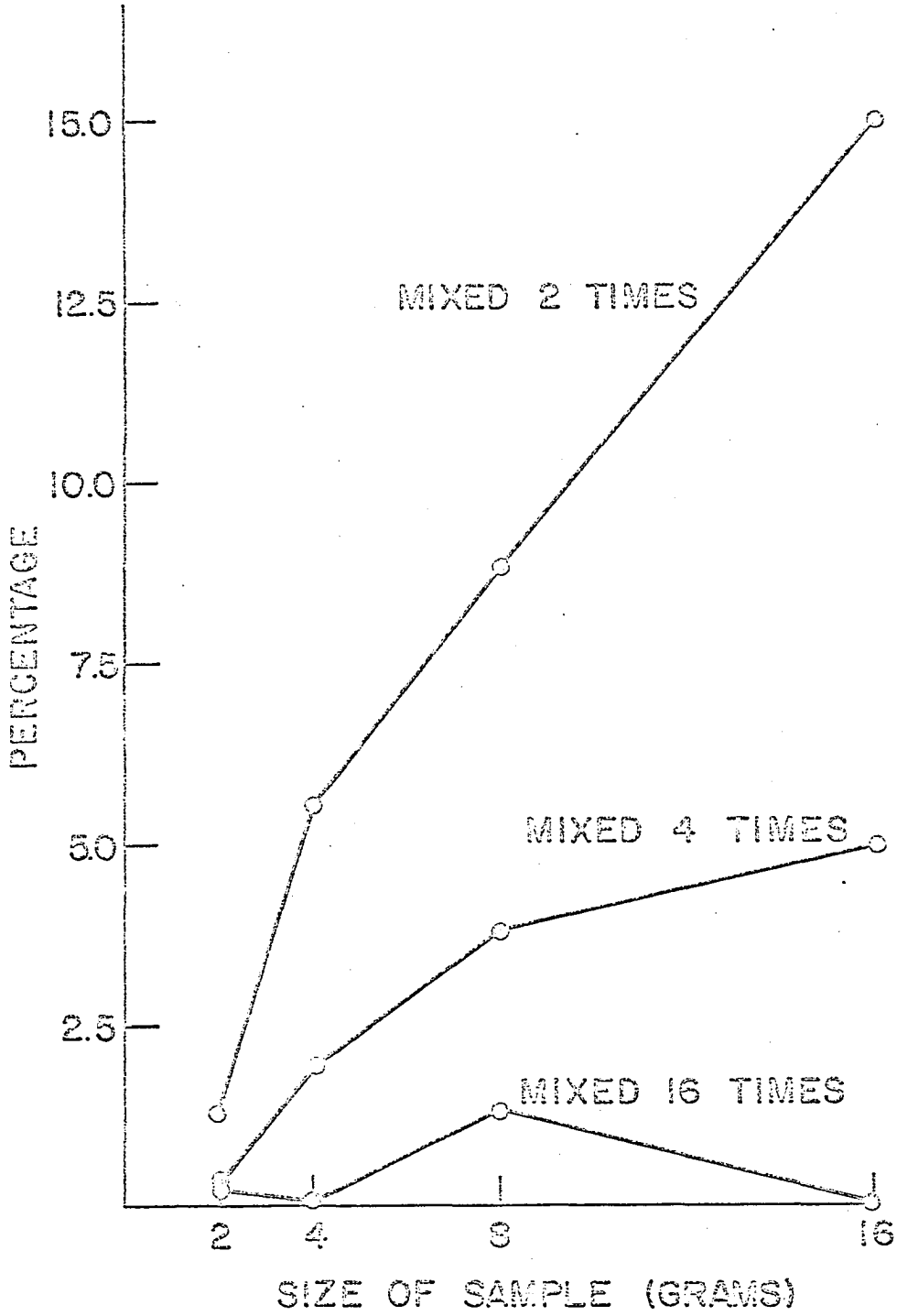


Figure 3. Experiment 1. Percentage of samples of different sizes containing red-stained alfalfa seeds in amounts which exceed Federal tolerance limits; unstained alfalfa seed substrate; from 160-gram batches of different degrees of uniformity

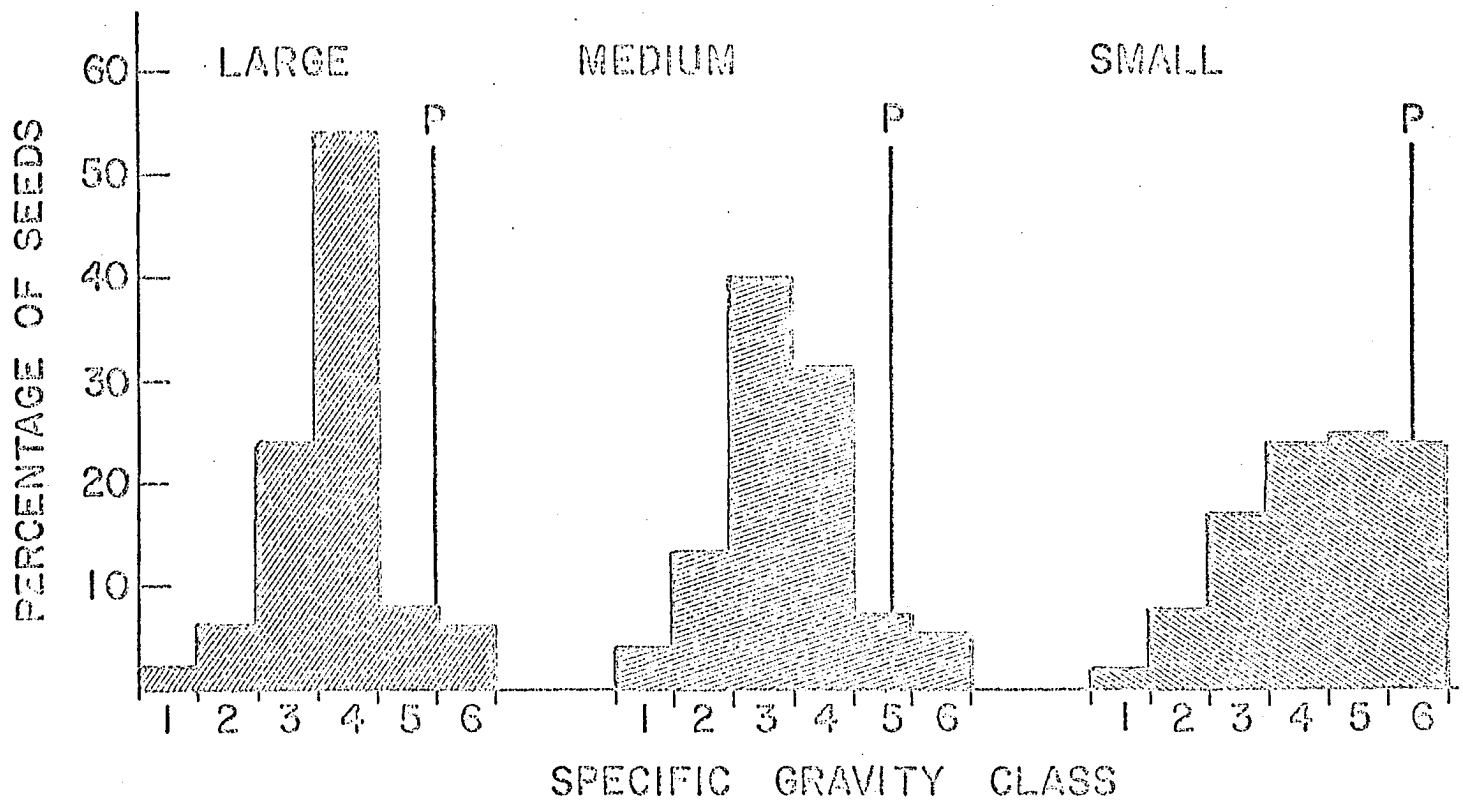


Figure 4. Experiment 2. Specific gravities of individual rape seeds of different sizes from a commercial rape seed lot; size classes described in Table 4. Specific gravity classes: 1, under 1.00; 2, 1.00 to 1.025; 3, 1.026 to 1.050; 4, 1.051 to 1.075; 5, 1.076 to 1.100; 6, over 1.100. P designates specific gravity reading obtained with air comparison pycnometer

Table 4. Experiment 2. Percentage of seeds of different sizes in a commercial lot of rape seed

Seed size class	Sieve size	Percentage of lot in size class
Large	Over 1/12" round-holed sieve	1.93
Medium large	Through 1/12" sieve; over 4/64" x 3/4" sieve	10.37
Medium	Through 4/64" x 3/4" sieve; over 10 x 10 mesh sieve	68.35
Medium small	Through 10 x 10 mesh sieve; over 1.651 mm. round-holed sieve	17.24
Small	Through 1.651 mm. sieve	2.06

(1) Variance values were plotted on graph paper. Observation of the location of the plotted points revealed no substrate differences.

(2) a. Bartlett's test for homogeneity of variance (31, p. 285) was applied to variance values of the 6 replications of each substrate. Results indicated in each case that the estimates of variance made in the 6 replications of each treatment did not differ significantly.

b. An F test was made to compare pooled variance values for all possible pairs of substrates. None of the tests revealed differences among substrates.

(3) The procedure outlined in (2) was repeated using variance values obtained from transformed data. The

Table 5. Experiment 2. Variance of numbers of indicator seeds[a] in five rape seed substrates. Batches mixed 3 times (after addition of indicator seeds) before sampling[b]

Replication	Substrate[c]				
	1	2	3	4	5
I	47.84	49.61	39.04	79.52	41.12
II	44.22	35.19	43.68	53.16	48.73
III	72.93	24.24	23.88	44.48	30.16
IV	21.62	36.48	59.20	43.14	43.79
V	36.72	79.79	53.52	27.22	56.34
VI	39.68	78.17	35.46	42.69	48.16
Pooled	42.05	50.09	40.72	46.37	42.85

[a]Rape seeds; specific gravity 1.025 to 1.075; graded in cupric chloride solutions. Bleached and stained indicator seeds were used with Substrates 1, 2, 4, and 5; black indicator seeds were used with Substrate 3.

[b]Mean number of indicator seeds per sample was 40.

[c]Substrate 1: Ungraded seed from the original rape seed lot; black.
Substrate 2: Medium size (through 4/64" x 3/4" sieve, over 10 x 10 mesh sieve); ungraded for specific gravity; black.
Substrate 3: Medium size; ungraded for specific gravity; yellow (bleached).
Substrate 4: Ungraded for size; medium specific gravity (1.025 to 1.075; graded in supric chloride solutions); black.
Substrate 5: Medium size; medium specific gravity; black.

square root transformation was made in accordance with recommendations of Cochran (3). No differences among substrates were found. Slightly smaller Chi square values were obtained when Bartlett's test was applied to the transformed values than when the test was applied to the original data.

Experiment 3

More declarations of heterogeneity occurred when samples from the poorly mixed population (batches mixed 2 times) were large than when samples were small (Table 6, 26, 27, 28). Furthermore, more heterogeneity declarations occurred for groups of 20 samples than for groups of 10 or 5 samples.

Sample size appeared to have little effect upon results of homogeneity tests when samples were drawn from a well-mixed population (batches mixed 16 times). In this population, more heterogeneity declarations occurred when homogeneity tests were made on groups of 5 samples than on groups of 10 or 20 samples.

The intermediate group size, 10, provided the most heterogeneity declarations when samples were drawn from the population which had been mixed 4 times. Sample size appeared to have little effect on results of homogeneity tests in this population.

The Leggatt homogeneity test was the most severe of the homogeneity tests compared, but the H homogeneity test was also severe (Tables 26, 27, 28). The Long and Short homogeneity tests resulted in only a few declarations of heterogeneity, and nearly all of those occurred with 16-gram samples; none occurred in 2-gram samples.

Table 6. Experiment 3. Heterogeneity declarations (10 possible); Leggatt homogeneity test applied to numbers of blue-stained alfalfa seeds[a]

Number of times mixed	Sample size (grams)	No. of samples per group			Total (120 possible)
		5	10	20	
2	2	0	0	2	2
	4	3	3	4	10
	8	2	6	8	16
	16	2	8	10	20
	Total (40 possible)	7	17	24	
4	2	1	2	2	5
	4	1	3	0	4
	8	1	3	0	4
	16	1	3	0	4
	Total (40 possible)	4	11	2	
16	2	1	0	1	2
	4	1	0	0	1
	8	1	0	0	1
	16	1	0	0	1
	Total (40 possible)	4	0	1	

[a]Data for the Long, Short, and H homogeneity tests are given in Tables 26, 27, and 28.

Variance of the mean number of blue-stained alfalfa seeds per gram[1] is shown in Table 7 for each group size-sample size-mixing treatment combination of Experiment 3. In poorly mixed seed (batches mixed 2 times), variance of the mean was greater

[1]Seed sampling is conducted primarily to obtain an estimate of the composition of a seed lot. In the problem at hand, an estimate is needed for the mean number of indicator seeds per unit weight. Variance of the mean is a statistic which measures the precision with which the true mean was estimated when testing was done using each of the different sample size group size-mixing treatment combinations. A low variance indicates that the true mean has been estimated with a large degree of precision.

Table 7. Experiment 3. Variance of the mean number of blue-stained alfalfa seeds per gram[a]; unstained alfalfa seed substrate; 10 replications

Number of times mixed	No. of samples per group	Sample size (grams) 2	4	8	16
2	5	.10	.15	.16	.17
	10	.05	.07	.09	.12
	20	.03	.04	.05	.06
4	5	.10	.10	.11	.07
	10	.05	.06	.06	.06
	20	.03	.02	.03	.03
16	5	.11	.11	.10	.09
	10	.05	.05	.04	.04
	20	.03	.02	.02	.02

[a]Means were: 4.570 seeds per gram for batches mixed 2 times, 4.522 seeds per gram for batches mixed 4 times, and 4.611 seeds per gram for batches mixed 16 times.

when samples were large than when they were small. This was true for all group sizes. In contrast, when batches had been mixed 4 or 16 times, variance of the mean was essentially the same for all sizes of samples included in the study.

In all three populations (batches mixed 2, 4, and 16 times), regardless of sample size, variance of the mean was less when groups consisted of 20 samples than when they consisted of 10 samples, and variance of the mean was less when there were 10 samples than when there were 5.

Results indicate that in well-mixed seed, the true mean was estimated with greater precision of samples per group were increased than when sample sizes were increased.

Experiment 4[1]

Effect of mixing treatment

Variance of counts of indicator seeds in rape seed batches which had been mixed but once was greater than variance of counts in batches which had been mixed 2 times; variance in batches mixed 2 times was greater than that in batches mixed 3 times or more; but mixing beyond the third time had no appreciable effect on variance. Variance differences due to mixing treatments were evident for all indicator seed concentrations which were tested (Table 29).

Effect of indicator seed concentration

Calculated variances of numbers of indicator seeds in samples from well-mixed batches (mixed 3 times or more) were approximately equal to the mean number of indicator seeds per sample (Table 8)[2]. In poorly mixed seed (mixed 1 or 2 times), variance exceeded the mean, often considerably. The ratio of variance to the mean in poorly mixed seed was low when concentration of indicator seeds was low (mean = 5), and was high when indicator seed concentration was high (mean = 40; Table 8).

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[1] Data obtained from Experiment 4 are given in Tables 29, 30, 31.

[2] The variance is equal to the mean in the Poisson distribution. The ratio of the variance to the mean provides an estimate of the degree to which experimental data depart from the Poisson distribution. Tolerance tables (Appendix E) are based on the assumption that numbers of weed seeds in a crop seed substrate are distributed in accordance with the Poisson distribution.

Table 8. Experiment 4. Ratio of pooled variance to mean number of indicator seeds present in four different concentrations in 16-gram samples, 20 samples per batch

Number of times mixed	Mean No. of indicator seeds per sample			
	5	10	20	40
1	1.44	2.42	4.20	5.44
2	1.34	1.26	1.37	1.92
3	0.88	0.92	1.17	1.05
4	0.72	1.08	0.93	0.95
8	0.88	1.00	1.15	1.15
16	0.97	0.77	1.19	0.92

Effect of sample size

Indicator seed count variances in samples of different sizes were not significantly different when mean number of indicator seeds per sample was held constant (Table 9).

Table 9. Experiment 4. Pooled variance of numbers of stained rape indicator seeds in samples from 320-gram batches; unstained rape seed substrate[a]

Ave. No. indicator seeds per sample	Sample size[b]			
	4 grams	8 grams	16 grams	32 grams
10	12.60	10.76	12.57	11.35[c]
20		27.95	27.42	31.56[c]
40			76.72	72.50[c]

[a]Data for individual replications are recorded in Table 29.

[b]Numbers of samples per batch were 80,40,20, and 10 when sample sizes were 4,8,16, and 32 grams, respectively. All batches were mixed 2 times before sampling.

[c]Differences among values on same line are not statistically significant.

Effects of mixing treatment, indicator seed concentration, and sample size upon variance of indicator seed counts in a rape seed substrate are summarized in Figure 5.

Comparison of homogeneity tests

The Leggatt homogeneity test led to the most declarations of heterogeneity when the different homogeneity tests were applied to data of Experiment 4 (Tables 10, 30). The H homogeneity test was less severe than the Leggatt homogeneity test, but was more severe than either the Long or the Short homogeneity test. Severity of all four tests was closely related to indicator seed concentration (Table 10); the greatest number of heterogeneity declarations occurred when indicator seed concentration was greatest.

Individual observations which exceeded tolerance limits

Percentages of samples which contained indicator seeds in numbers which exceeded tolerance limits of the Federal Seed Act (Appendix E) are recorded in Table 31. A much higher percentage of observed values exceeded tolerance when indicator seed concentration was high than when it was low in poorly mixed seed (Figure 6).

Experiment 5

There were no significant differences after mixing among variances of indicator seeds which had been placed in different positions prior to mixing (Table 11). Pooled variances of

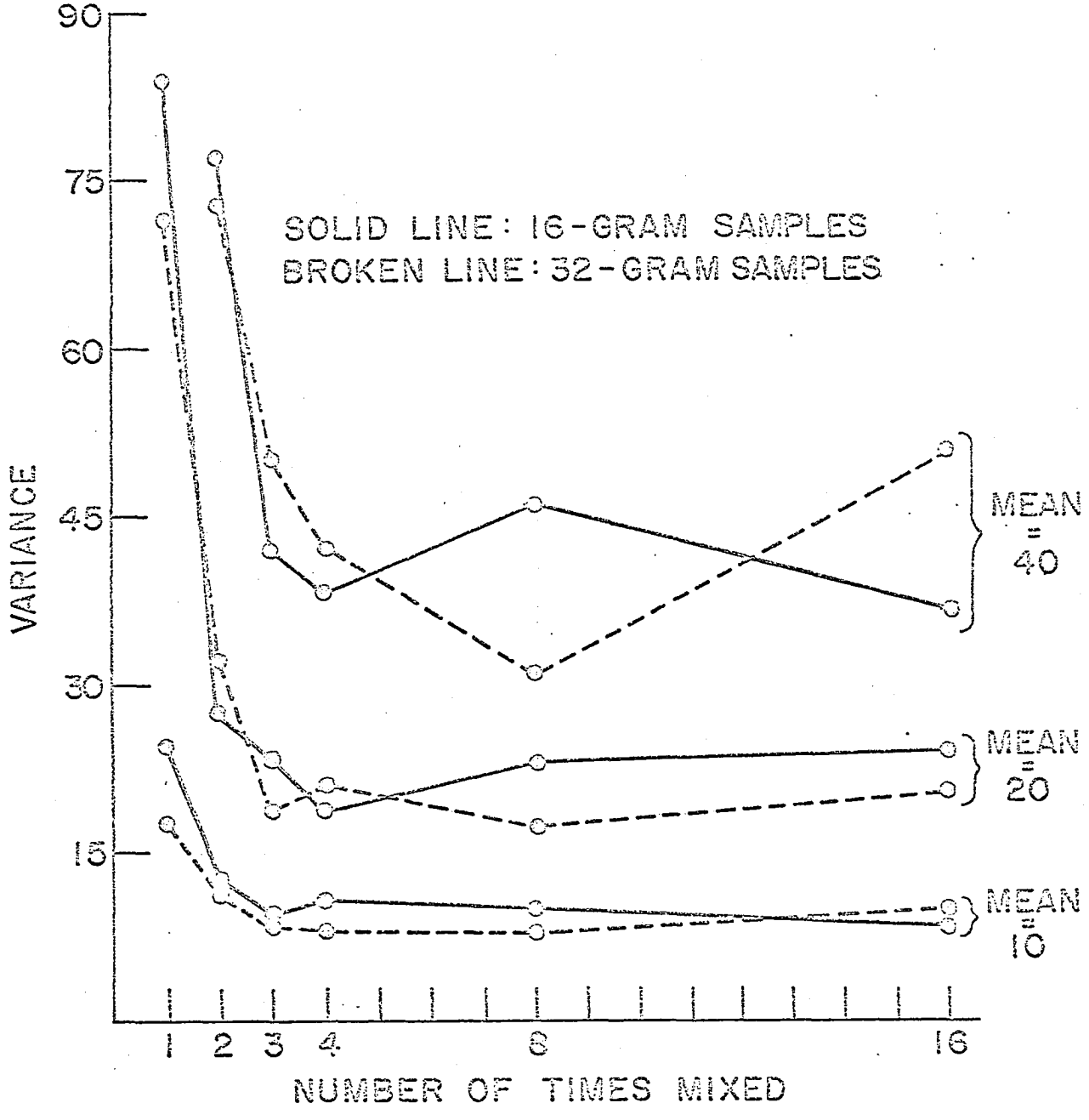


Figure 5. Experiment 4. Variance of numbers of yellow-stained rape seeds in samples from 320-gram batches; unstained rape seed substrate

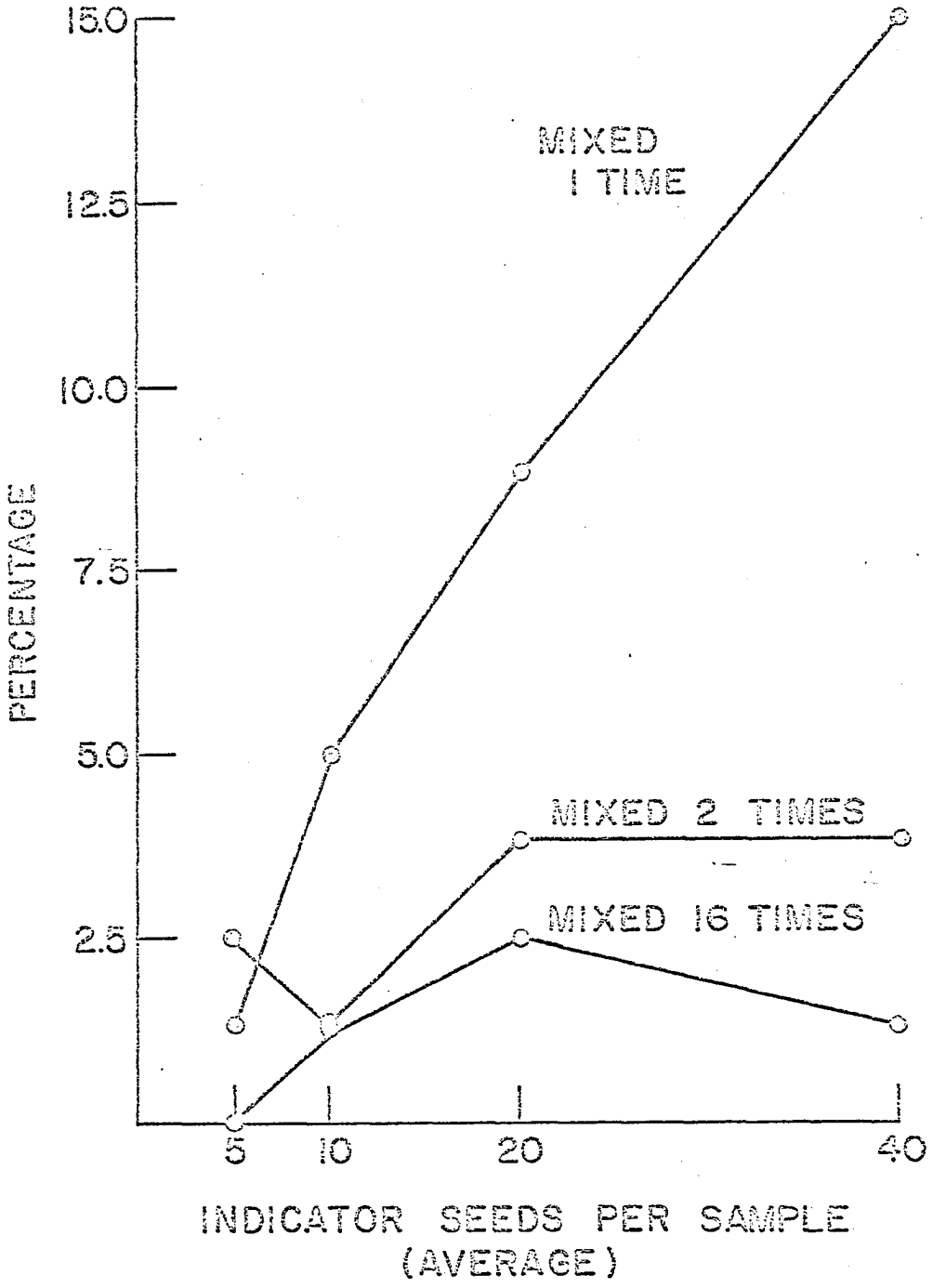


Figure 6. Experiment 4. Percentage of 16-gram samples containing indicator seeds in amounts which exceed Federal tolerance limits; yellow-stained rape seeds in an unstained rape seed substrate

numbers of indicator seeds present in sample from batches mixed 2 times were significantly greater than variances derived from batches mixed 3 times.

Table 10. Experiment 4. Comparison of homogeneity tests (summary). Yellow-stained rape seeds in unstained rape seed substrate. Data presented in terms of heterogeneity declarations (48 possible)[a]

Mean No. of indicator seeds per sample	Test applied				Total (192 possible)
	Leggatt	"H"	Short	Long	
10	7	5	1	0	13
20	15	8	8	8	39
40	23	14	9	11	57
Total (144 possible)	45	27	18	19	

[a]Combined data for all mixing treatments and for 16- and 32-gram samples. Detailed data are given in Table 30.

Table 11. Experiment 5. Effect of starting location of indicator seeds in mixing apparatus on variance after mixing of numbers of stained alfalfa seeds in 8-gram samples from 160-gram batches; unstained alfalfa seed substrate[a]

Starting position	Replication	No. of times mixed	
		2	3
1	I	30.41	46.31
	II	30.22	42.30
	III	35.31	22.96
	IV	71.53	27.75
	Pooled	40.33	33.69
2	I	25.89	43.94
	II	33.74	29.73
	III	92.78	34.58
	IV	41.69	46.69
	Pooled	46.75	37.70
3	I	51.04	30.88
	II	67.43	18.87
	III	51.29	21.57
	IV	54.32	27.94
	Pooled	53.91	23.89
4	I	48.13	35.40
	II	26.68	20.96
	III	72.11	34.12
	IV	33.94	41.47
	Pooled	43.58	32.27

[a]Mean number of indicator seeds per sample was 20.

DISCUSSION AND CONCLUSIONS

Mixing Characteristics of Different Kinds of Indicator Seeds

Results of Experiment 1 (Figure 2) are in agreement with Leggatt's findings (15, 16, 17, 18, 20) that different kinds of seeds exhibit different mixing characteristics. Leggatt (21; Appendix F) described a procedure whereby tolerances for foreign seeds can be adjusted with respect to mixing patterns. This procedure requires a separate tolerance calculation for each seed kind exhibiting an unusual mixing pattern. An alternate procedure would be to leave the tolerance tables in their present form making adjustments instead as to labeled numbers of foreign seed kinds. Tolerance tables would then apply equally to all seed kinds. Thus the procedure could be put into use without the necessity of legislation approving tolerance table changes. A way in which this can be done is outlined by the present author in Appendix F.

Comparison of Homogeneity Tests

Heterogeneity declarations

Four homogeneity tests were compared using data derived from the present study (Tables 3, 10, 20, 21, 22, 23, 24, 26, 27, 28, 30). The Leggatt homogeneity test provided the greatest number of heterogeneity declarations under nearly all testing conditions. Performance of the four tests can be

summarized as follows:

Homogeneity test	Total No. of tests made	Heterogeneity declarations	
		Number	Percentage
Leggatt	1039	431	41.5
H	1039	231	22.2
Long	1039	110	10.6
Short	827	102	12.3

I have adopted a pragmatic criterion for comparing these homogeneity tests. Data from Experiments 1 through 5 were divided into two categories: lots in which less than 5% of the observations (pooled data from all replications of identical treatments) exceeded tolerance limits (homogeneous lots) and lots in which 5% or more of the observations exceeded tolerance limits (heterogeneous lots).

Comparative performances of the four tests are summarized in Table 12. The Long and Short homogeneity tests seldom led to wrong declarations of heterogeneity (Type I errors), but both tests often failed to declare lots heterogeneous when in fact they were heterogeneous (Type II errors).

The Leggatt homogeneity test is too severe in its present form (Table 12). Reduction of Type I errors could be made by changing the probability level for the Figure of Homogeneity from 5% to 1%. However, this would increase the percentage of Type II errors. The reverse situation exists for the H homogeneity test. The critical value of H could be lowered (say

Table 12. Experiments 1 through 5. Comparison of homogeneity tests. Type I and Type II errors

Category of seed lots	Homogeneity test applied	Percentage of samples which were declared heterogeneous	Percentage of errors made	
			Desired maximum	Obtained
			% Type I[a]	%
Homogeneous	Leggatt	17.6	5	17.6
Less than 5% of all observations exceeding tolerance limits[b]	H	6.4	5	6.4
	Long	1.7	5	1.7
	Short	1.9	5	1.9
			Type II[c]	
Heterogeneous	Leggatt	90.7	5	9.3
5% or more of all observations exceeding tolerance limits[b]	H	54.7	5	45.3
	Long	31.0	5	69.0
	Short	28.5	5	71.5

[a]Type I errors were made when samples were declared heterogeneous on basis of test although, in fact, the samples had been drawn from homogeneous lots.

[b]Tolerance values determined on basis that mean number of indicator seeds per sample was the number labeled or represented (columns 1, 3 of Appendix E).

[c]Type II errors were made when samples were declared homogeneous although, in fact, the samples had been drawn from heterogeneous lots.

from 2.00 to 1.80), thus lowering the percentage of Type II errors. However, this beneficial effect would be offset by a raising of Type I errors.

A weakness of present tests

Results of Experiment 1 (Table 3) and Experiment 3 (Table 6; 8- and 16-gram samples from batches mixed 2 times) confirmed the findings of Westmacott and Linehan (35) that imperfectly mixed seed lots are more likely to be declared heterogeneous when tested by the Leggatt homogeneity test if samples are large and if many samples are tested than if samples are small in size and number. The H, Long, and Short homogeneity tests also exhibit this characteristic (Tables 3, 30). Further study (Experiment 4; Table 10) revealed that the apparent influence of sample size upon homogeneity test results is due to the fact that the homogeneity tests are more sensitive when applied to large numbers than when applied to small numbers; large samples contain more indicator seeds than small samples.

The significance of this finding is as follows: if tested by the Leggatt, H, Long, or Short homogeneity test, a lot may be declared homogeneous with respect to one seed kind, but heterogeneous with respect to another seed kind, merely because there are more seeds of the first kind present in each sample!

Inter-relationship of Leggatt, H, and Long homogeneity tests

The Leggatt, H, and Long homogeneity test statistics can all be expressed in terms of the statistic which Westmacott and Linehan (35) defined as

$$h = \frac{\text{Observed variance}}{\text{Theoretical minimum variance}} = s^2/\sigma^2 .$$

If a Poisson distribution is assumed,

$h = s^2/\sigma^2 = s^2/\bar{x}$, where $\bar{x}$ = the sample mean.

The H statistic was defined by Miles (24) as

$$H = \frac{\text{Observed variance}}{\text{Theoretical minimum variance}} - 1 = s^2/\sigma^2 - 1.$$

Therefore, $H = h - 1$.

Leggatt's Figure of Homogeneity (Appendix A) can be expressed as

$$\chi^2_{n-1} = \Sigma\frac{(\text{Observed} - \text{Expected})^2}{\text{Expected}} = \frac{\Sigma(x-\bar{x})^2}{\bar{x}}$$

where n = the number of counts made, and the subscript, n-1, refers to the number of degrees of freedom associated with χ^2.

Anderson and Bancroft (1, p. 80) have shown that $(n-1)s^2/\sigma^2$ is distributed as χ^2 with (n-1) degrees of freedom. Assuming a Poisson distribution,

$$\chi^2_{n-1} = (n-1)\ x^2/\sigma^2 = (n-1)\ x^2/\bar{x} = (n-1)h.$$

Miles, Carter and Shenberger (25) defined the computed

value of F for the Long homogeneity test, non-chaffy seed, as

$$F = s^2/1.69\sigma^2 .$$

For the Poisson distribution,

$$F = (1/1.69)\ s^2/\bar{x} = (1/1.69)\ h,$$

(n-1) and infinity degrees of freedom.

To summarize:

$$h = \frac{\text{Figure of Homogeneity}}{(n-1)} = H \div 1 = 1.69F.$$

Critical h values[1]

Critical values of the Figure of Homogeneity, H, and F, expressed in terms of h, are tabulated in Table 13 for tests of 5, 10, and 20 samples. Critical h values for the Leggatt homogeneity test and the Long homogeneity test are smaller when tests comprise large numbers of samples than when tests are of few samples (Table 13). In contrast, critical h values for the H homogeneity test remain constant regardless of how many samples are tested. Consequently, we should be able to reject at least one of the homogeneity tests on this basis alone.

Experimental data (Table 14) indicate that the H test is to be preferred to the Leggatt or Long homogeneity tests for distinguishing between degrees of uniformity. In imperfectly mixed seed (mixed 2 times), values of h were approximately equal when calculated from tests of 5, 10, and 20 samples (Table 14). The phenomenon of low h values occurring with large numbers

[1]The critical value of a statistic is the maximum value which the statistic may have for a homogeneity declaration to be made; any higher value of the statistic would result in a declaration of heterogeneity.

Table 13. Effect of sample number on critical h values for the Leggatt, H, and Long homogeneity tests[a]

Number of samples per test	Critical h value Test applied		
	Leggatt	H	Long
5	2.37	2.00	4.01
10	1.88	2.00	3.18
20	1.59	2.00	2.67

[a] $h = \frac{\text{Observed variance}}{\text{Theoretical minimum variance}}$. The critical value is the value of h above which a lot will be declared heterogeneous. Values in this table were calculated from the equation:

$$h = \frac{\text{Figure of homogeneity}}{(n-1)} = H \div 1 = 1.69\ F.$$

Table 14. Experiment 3. Effect of sample number upon calculated[a] values of h for numbers of blue-stained alfalfa seeds in samples from 160-gram batches; unstained alfalfa seed substrate

Number of samples per test	Number of times mixed	Mean No. of indicator seeds per sample			
		9.5[b]	19[c]	38[d]	76[e]
5	2	1.15	1.61	1.75	1.79
10	2	0.97	1.41	1.92	2.65
20	2	1.23	1.50	1.93	2.64
5	4	1.01	1.07	1.18	0.71
10	4	1.16	1.28	1.37	1.23
20	4	1.34	1.04	1.09	1.19
5	16	1.21	1.15	1.04	0.92
10	16	1.06	0.97	0.98	0.78
20	16	1.04	1.02	0.88	0.79

[a] Calculated from pooled data of 10 replications.

[b] 2-gram samples.

[c] 4-gram samples.

[d] 8-gram samples.

[e] 16-gram samples.

of samples (as is consistent with critical values of the Leggatt and F homogeneity tests; Table 13) was apparent only when samples were drawn from batches mixed 16 times. The implication is this: the Leggatt test will separate well mixed lots from poorly mixed lots, but it will not distinguish between degrees of imperfection. Experiences reported by Westmacott and Linehan (35) and by Linehan and Mathews (22) indicate that a seed homogeneity test is needed which will distinguish between degrees of imperfection.

Recommended Procedures for Testing Seed for Homogeneity with Respect to Foreign Seeds

Data which have been presented indicate that none of the homogeneity tests that are presently available fulfill the need of the seed industry. A satisfactory homogeneity test can be made available in one of two ways: (1) modification of one of the tests that is presently available, or (2) development of a new test. Both alternatives will now be considered.

Modification of a present homogeneity test

Selection of one homogeneity test The Long and Short homogeneity tests are entirely unsatisfactory in their present forms because both tests result in excessive numbers of Type II errors (Table 12). The Short homogeneity test is a crude test at best, since the statistic employed (range of foreign seed counts) is dependent upon only two observations, the high

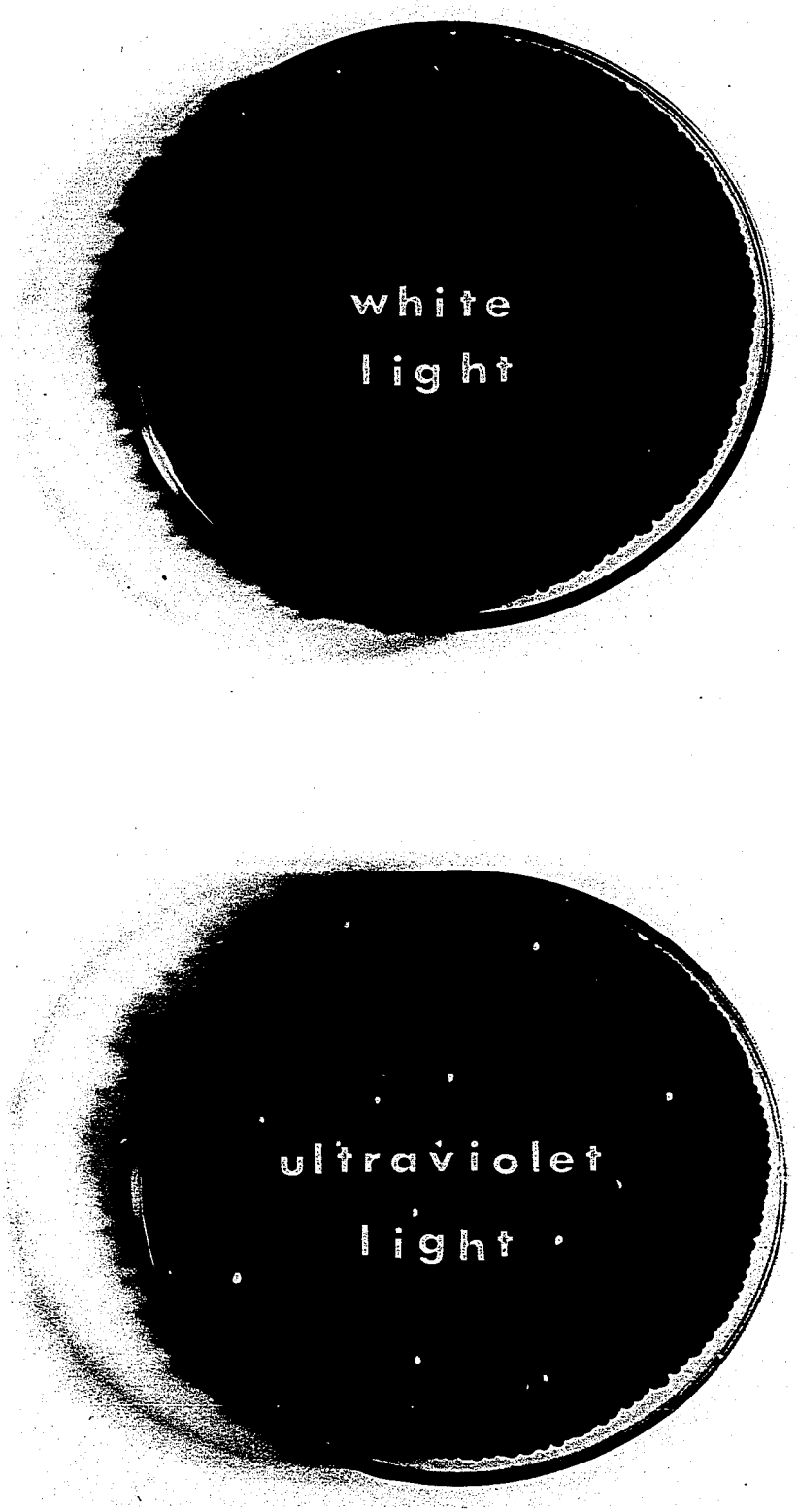
and low counts. The Long homogeneity test could probably be made into a satisfactory test[1]; however, even after revision, the Long homogeneity test would be no better than the Leggatt or H homogeneity tests. Neither the Long homogeneity test nor the Short homogeneity test appear to be in use by the seed industry. It is recommended that they be dropped from further consideration.

Consideration of the data of Tables 13 and 14 leads to rejection of the Leggatt homogeneity test. Only the H test remains.

Recommended modifications in the H test

Use of indicator seeds Indicator seeds should be used when seed is tested for homogeneity. Indicator seeds may be marked in many ways. Staining of seeds is required by the Federal Seed Act for imported seed of red clover and alfalfa seed (34, Section 201.104). Therefore, procedures for staining have already been developed. Possible objections relating to the effect of stained seeds upon appearance of seed lots can be avoided through the use of stains visible only when viewed under an ultraviolet (black) light (Figure 7). Radio-isotopes have been used in the blending of liquids (9), and their use could be considered for tagging indicator seeds.

[1]Calculations, using data of Experiment 4, $\bar{x} = 20$, $n = 20$, indicate that the statistic for the Long homogeneity test would be more useful if defined as $F = s^2/0.90\bar{x}$ (present definition: $F = s^2/1.69\bar{x}$).


white
light
ultraviolet
light

Concentration of indicator seeds The necessary indicator seed concentration is dependent upon the composition of the lot being tested. Since the value of the h statistic (and hence the H statistic) varies with concentration in poorly mixed seed lots (Table 8), indicator seed concentration should be adjusted to a level exceeding that of foreign seed kinds present. Data of Experiment 4 (Table 30) indicates that if a mixing treatment produced batches which were homogeneous with respect to indicator seeds at one concentration, the same mixing treatment produced batches which were homogeneous with respect to indicator seeds at all lesser concentrations.

Number of samples per test At least 20 samples should be included in each homogeneity test. Tests of 20 samples in Experiment 3 were superior to tests of 5 or 10 samples for distinguishing between poorly mixed and well-mixed seed lots (Table 6). Until additional data are available concerning expected percentages of Type I and Type II errors from this modified test, it is recommended that at least two tests (each of 20 samples or more) be made. If both tests provide the same answer concerning homogeneity of the lot, no further sampling should be necessary. If there is a discrepancy in test results from the two samples, one or two more tests should be made.

Sample size Total amount of seed in each sample is relatively unimportant so far as indicator seed distribution

is concerned (Table 9); however, until more experience has been gained, a reasonable sample size would appear to be that size which will be tested for inspection purposes after the seed has been labeled.

Critical H values Critical H values are necessarily different for each indicator seed concentration. Adjustment of the critical H value for concentration corrects the weakness of the H test as it is now described (Appendix D). Critical values of H suggested by the data of Tables 8 and 14 are as follows:

Mean number of indicator seeds per sample	Critical H value
20	0.50
40	0.90

Development of a new homogeneity test

Basis for the test All of the homogeneity tests herein described provide indirect predictions of the desired information: the probability that a sample drawn from the lot will contain numbers of foreign seeds which exceed tolerance limits. A direct procedure for obtaining the desired information will now be described.

Description of the Direct homogeneity test

Definitions

Indicator seed An indicator seed is one differing sufficiently from substrate seeds to allow easy detection.

Heterogeneous seed lot A heterogeneous seed

lot is one from which numbers of foreign seeds in random samples will exceed tolerance limits 5% or more of the time.

Homogeneous seed lot A homogeneous seed lot is one from which numbers of foreign seeds in random samples will exceed tolerance limits less than 5% of the time.

Primary sample[1] When a seed lot is sampled, either in containers or in bulk, several individual samples are drawn from different containers or different places in the bulk. Each probe of seed or each handful is called a primary sample.

Composite sample[1] All the primary samples are combined in a suitable container (bag, box, tray, etc.). These combined primary samples are called the composite sample. This sample is usually much larger than required for the different tests and consequently it must be reduced.

Submitted sample[1] When the composite sample has been properly reduced it is called the submitted sample. This sample is submitted to a testing station for quality tests.

Working sample[1] The term working sample means the reduced sample, obtained from the submitted sample, on which one of the quality tests is made.

Hypothesis[2] If a seed lot is homogeneous with respect to an indicator seed kind present in any amount up to

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[1]These definitions were taken from the International rules for seed testing (12, Sections 2.2.2, 2.2.3, 2.2.4, and 2.2.5).

[2]Based upon experimental findings of this thesis.

1%, then the seed lot is also homogeneous with respect to all other seed kinds which are physically similar to the indicator seed kind and are present in any amount equal to or less than that of the first indicator seed kind.

Procedure Adjust indicator seed concentration to a level above that of the kinds of foreign seeds present in the lot. Use a high enough concentration of indicator seeds to insure that there are an average of 20 or more indicator seeds present per working sample.

Draw 20 primary samples at random from the lot. Obtain a working sample from each primary sample. Count number of indicator seeds per working sample. Calculate average number of indicator seeds per sample. Obtain the maximum number of seeds within tolerance of the average from tolerance table (Appendix E). Determine the number of samples which contain numbers of indicator seeds which exceed the tolerance limit. If 12 or more samples contain excess numbers of indicator seeds, declare the lot heterogeneous.[1] If less than 12 samples contain excess numbers of indicator seeds, obtain another 20 working samples (each from a primary sample) and determine the number of indi-

[1]The number 12 is an estimate which is made using the assumptions that (1) the numbers of samples which contain indicator seeds in excess of tolerance are distributed according to the Poisson distribution, and (2) that the tolerance table of Appendix E, which is intended for use with numbers of seeds, can be applied to these numbers of samples. Experience may lead to modification of these assumptions.

cator seeds in each. Calculate the average number of indicator seeds per sample, using counts from all 40 samples tested. If 12 or more of the 40 samples contain numbers of indicator seeds in excess of tolerance, declare the lot heterogeneous; otherwise obtain and examine another 20 samples. Repeat the procedure until 100 samples have been examined. If less than 12 samples out of 100 contain excess numbers of indicator seeds, declare the lot homogeneous.

Adjustment for cluster effect When it is suspected that one or more of the seed kinds in the seed lot exhibit the cluster effect (Appendix F), an estimate of cluster size can be made on the basis of a single test of 20 or fewer working samples. Adjust labeled number of weed seeds for cluster size as shown in Appendix F.

Labeling Following completion of the homogeneity test, working samples and the remaining portions from primary samples can be combined to form a composite sample for the lot. The composite sample may be reduced to a submitted sample. The analysis to meet labeling requirements can be made on a working sample drawn from this submitted sample.

Testing Seed for Homogeneity with Respect to Purity or Germination Percentages

Very little data were obtained for indicator seeds present in a concentration which exceeded 1%. On the basis of findings in the present study, it is hypothesized that indicator seeds in concentrations of 1% or less can be used to measure homogeneity of a lot with respect to pure seed and/or germination percentages. Proof, or disproof, of this hypothesis will require additional research.

Sampling and Counting for Homogeneity Tests

I have made recommendations for sampling and counting indicator seeds in as many as 100 samples for a single homogeneity test. Homogeneity testing obviously requires more data than is required for determination of average foreign seed concentration of a lot; no doubt the use of such tests may be impractical in many marketing situations. But the task is not unrealistic with present equipment and analytical personnel, viz.:

(1) Automatic samplers are presently in use; obtaining 100 samples from the production line should not be especially difficult.

(2) The counting of 20, 30, or 40 indicator seeds in a sample requires only a fraction of the time that is required to make a complete purity analysis. An ex-

perienced seed analyst should be able to count indicator seeds (20 indicator seeds per sample average) in 100 samples in less than 4 hours. This time can be shortened by the use of automatic counting devices. Radioisotopes have been used in the blending of liquids (9), and the use of isotopes should be considered for tagging indicator seeds.

Present and Potential Uses of Homogeneity Tests

(1) Homogeneity tests can be used by seedsmen and law enforcement officials. The extent of their employment will be largely controlled by the size and value of seed lots in relation to the cost of making the tests. Homogeneity testing of small seed lots or low unit value seed kinds may never be practical. However, it appears reasonable to suggest that homogeneity tests should be made occasionally in every seed processing plant to check on procedures which are assumed to be satisfactory. Most disputes between seedsmen and law enforcement officials concerning seed labeling are probably due to insufficient mixing of the seed lots involved rather than to deliberate misrepresentation of the seed.

(2) Homogeneity tests can be employed to compare blending procedures and equipment. Homogeneity tests should

make it possible for seedsmen to determine: "What is the best blending method for this kind of seed?", "What is the maximum size lot which can efficiently be blended in this processing plant?", and similar questions. Improvements in blending procedures might reduce the necessity for homogeneity determinations of individual seed lots.

(3) Homogeneity tests could play a vital role in evaluation and improvement of blending equipment design.

(4) Blending problems are encountered in a variety of commodities besides agricultural seed. The homogeneity tests which have been described in this thesis might prove useful in other industries subsequent to appropriate transliteration.

SUMMARY

This study was concerned with the evaluation of seed homogeneity tests. Batches of seed (approximately 73,000 seeds per batch) were mixed to varying degrees of uniformity. Variances for numbers of indicator seeds (seeds differing sufficiently from substrate seeds to allow easy detection) in samples from the batches were calculated. Homogeneity of batches with respect to indicator seeds was determined by the use of four homogeneity tests: (1) the Leggatt homogeneity test (which employs the statistic, Figure of Homogeneity = $[x - \bar{x}]^2/\bar{x}$), (2) the H homogeneity test (H = [observed variance / theoretical minimum variance] - 1), (3) the Miles *et al.* "Long" homogeneity test (F = observed variance / [1.69] [theoretical minimum variance]), and the Miles *et al.* "Short" homogeneity test (range of counts).

The statistics employed in the Leggatt, H, and Long homogeneity tests were shown to be related: h = Figure of Homogeneity /(n-1) = H + 1 = (1.69) F, where h = observed variance / theoretical minimum variance.

All four tests are more sensitive when calculations are made with large numbers than with small numbers; therefore a lot could be declared homogeneous with respect to one seed kind but heterogeneous with respect to another seed kind merely because there were more seeds of the first kind present

in each sample. This phenomenon was noticed by previous workers, but was thought to be a function of sample size.

The four tests were compared on the basis of numbers of heterogeneity declarations made. The Leggatt homogeneity test led to the greatest number of heterogeneity declarations, and the Long and Short homogeneity tests were the least severe.

Since these gross comparisons tell nothing of the "correctness" of the tests, data were examined for each of the homogeneity tests to determine numbers of heterogeneity declarations made for batches that were "known" to be homogeneous and on batches "known" to be heterogeneous. Batches were defined as being homogeneous or heterogeneous on the basis of the percentage of samples from the batches which contained numbers of indicator seeds in excess of legal tolerance limits (less than 5% in homogeneous lots; 5% or more in heterogeneous lots). Percentages were calculated of Type I and Type II errors made by use of each of the four tests. Results indicated that the Long and Short homogeneity tests allowed far too many heterogeneous lots to pass as homogeneous (approximately 70% Type II errors). The Leggatt test resulted in too many declarations of heterogeneity in homogeneous lots (approximately 18% Type I errors), and in addition was not capable of distinguishing between different degrees of imperfection. The H statistic distinguished between imperfection degrees, but only when indicator seed concentrations in batches being com-

pared were identical.

The foregoing results indicate that none of the homogeneity tests, in their present form, fulfill the need of the seed industry. Recommendations are made for modifying the H homogeneity test. Key changes entail the use of indicator seeds (concentration set above concentration of foreign seeds present in the lot) and critical H values which vary with indicator seed concentration (critical H = 0.50 when $\bar{x} = 20$; critical H = 0.90 when $\bar{x} = 40$).

Also, a new test, the Direct homogeneity test, is proposed. This test makes a direct measurement of the percentage of samples from a lot which contain numbers of indicator seeds which exceed legal tolerance limits.

Experimental findings were in agreement with the following hypothesis: If a seed lot is homogeneous with respect to an indicator seed kind present in any amount up to 1%, then the seed lot is also homogeneous with respect to all other seed kinds which are physically similar to the indicator seed kind and are present in any amount equal to or less than that of the indicator seed kind.

The statistic, h, is equivalent to cluster size (c) as defined by C. W. Leggatt. Leggatt outlined a method of calculating tolerances, by using c, for kinds of seeds which follow irregular mixing patterns (i.e., which exhibit the "cluster effect"). The present author demonstrates a way in which

labeled numbers can be adjusted for foreign seed kinds which exhibit the cluster effect; following this adjustment, tolerance tables in their present form would apply equally to all weed seed kinds.

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ACKNOWLEDGMENTS

I wish to express a sincere "thank you" to the following persons who had a direct influence on this thesis:

Dr. Duane Isely, my major professor, has served as a counselor and friend through all phases of my graduate program at Iowa State. His help in the organization of the thesis writing has been invaluable.

Dr. L. E. Everson's interest in the topic aided me in making the selection of "Seed Homogeneity" as a thesis problem. He helped me with the planning of some of the experimental work, and he made many good suggestions concerning the thesis writing.

Dr. C. S. (Mike) Shih, a former graduate student in the Iowa State University Statistics Department, met with me on a weekly basis over a 6-8 month period. These sessions with Mike were a tremendous aid to me in determining which way I should go with the experimental work.

Dr. Don F. Grabe listened, through many a noon hour, to my explanations of experimental findings and to my plans for additional experiments. He provided many worthwhile suggestions which had a direct bearing on final conclusions of the thesis.

My wife, Elnor, and my children, Jill and Donn, have -- by being understanding, helpful, and sometimes just plain quiet-- helped me to complete this project.

APPENDIX A: LEGGATT HOMOGENEITY TEST[1]

Extract: Number of Weed and Crop Seeds[2]

To test for homogeneity of a seed lot with respect to number of weed and/or crop seeds (foreign seeds), make a number of analyses, N, of samples drawn at random from the lot. All samples must be of the same size.

Determine the Figure of Homogeneity as follows:

(1) Square the number of foreign seeds in each of the analyses and total the squares.

(2) Divide this figure by the mean number of foreign seeds.

(3) Subtract from the quotient the total number of foreign seeds in the N analyses.

The lot is not homogeneous if the Figure of Homogeneity is greater than the appropriate value given below.

Number of samples (N)	Limit for homogeneity
2	3.8
3	6.0
4	7.8
5	9.5
6	11.1
7	12.6
⋮	
31	43.8

[1]Originally described by Leggatt in 1952 (19). Included in the 1953, 1956, and 1959 International rules for seed testing (10, pp. 43-48; 11, pp. 44-49; 12, pp. 556-561).

[2]Instructions for determining homogeneity with respect to germination and purity have been omitted from this condensation.

APPENDIX B: THE LONG HOMOGENEITY TEST[1]

Extract: Number of Weed and Crop Seeds[2]

The Long homogeneity test is an F test. The F value is computed by dividing the variance of the samples by the maximum variance permitted in a "homogeneous" lot. If the computed F value exceeds the appropriate F value, the lot is declared heterogeneous.

No. of samples tested	F	
	5%	1%
5	2.37	3.32
6	2.21	3.02
7	2.09	2.80
8	2.01	2.64
9	1.94	2.51
10	1.88	2.41
:		
17	1.64	1.99
21	1.57	1.87
:		

Use the 1% probability level when average weed and/or crop seed counts are from 0 to 6 inclusive and the 5% probability level for counts over 6.

For nonchaffy seeds, computed F = sample variance/1.69 $\bar{x}$

For chaffy seeds, computed F = sample variance/3.24 $\bar{x}$

where $\bar{x}$ is the sample mean.

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[1]Condensed from paper by Miles, Carter and Shenberger (25).

[2]Instructions for determining homogeneity with respect to germination and purity have been omitted from this condensation.

APPENDIX C: THE SHORT HOMOGENEITY TEST[1]

Extract: Number of Weed and Crop Seeds[2]

Obtain 5, 10, or 20 samples from the lot to be tested. Analyze the samples separately; compute the average number of weed and/or crop seeds (foreign seeds) present. Declare the lot homogeneous if the observed range does not exceed the "maximum range for homogeneity" given below. Otherwise, declare the lot heterogeneous.

Average No. of foreign seeds per working sample	Nonchaffy seed			Chaffy seed		
	5	10	20	5	10	20
1.0	-	6	7	-	9	10
2.0	8	9	10	-	13	14
3.0	10	11	12	14	16	17
4.0	12	13	14	16	18	20
5.0	13	15	15	18	20	22
6.0	13	15	16	19	21	23
7.0	14	16	17	19	22	25
8.0	15	17	18	20	23	26
9.0	15	17	19	21	24	27
10.0	16	18	20	22	25	29
:						
19.0	21	25	28	30	35	39
20.0	22	26	29	31	36	40
:						
40.0	31	36	41	44	50	57
:						

[1] Condensed from paper by Miles, Carter and Shenberger (25).

[2] Instructions for determining homogeneity with respect to germination and purity have been omitted from this condensation.

APPENDIX D: THE H HOMOGENEITY TEST[1]

Extract: Number of Weed and Crop Seeds[2]

Sample no less than the following number of bags:

Number of bags in lot	Number of bags to sample
1 - 9	Every bag
10 - 15	10
16 - 25	12
26 - 35	15
36 - 49	17
50 - 64	20
65 - 80	23
81 -100	25
101 -120	27
over 120	30

Choose bags strictly at random. Draw a bag-sample from each chosen bag. The bag-sample must comprise small portions taken across the diameter of the bag at the top, middle and bottom. The weight of each bag-sample shall be not less than half the weight required when samples are submitted for purity analysis.

Draw a working sample of about 10,000 seeds from each bag-sample.

The lot may be checked for homogeneity with respect to any kind or kinds of weed and/or crop seed (foreign seeds) present. Count the number of foreign seeds in each working sample. Calculate the Heterogeneity Value (H).

$$H = (V / W) - 1$$

where V = sample variance
W = sample mean

Report H, W, number of working samples, weight of working samples, and number of bags in the lot.

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[1]Condensed from the 1966 International rules for seed testing (13, pp. 140-144).

[2]Instructions for determining homogeneity with respect to germination and purity have been omitted from this condensation.

APPENDIX E: NOXIOUS WEED SEED TOLERANCES[1]

201.65. Noxious-weed seeds in interstate commerce. Tolerances for rates of occurrence of noxious-weed seeds shall be recognized and shall be applied to the number of noxious-weed seeds found by analysis in the quantity of seeds specified for noxious-weed seed determinations in section 201.46 and section 201.52. Representations showing the rate of occurrence indicated in columns 1 and 3 will be considered within the tolerance if no more than the accompanying number in columns 2 and 4 are found by analysis in the administration of the act. Applicable tolerances are calculated by the formula, $Y=X+1+1.96\sqrt{X}$, where X is the number labeled or represented and Y is the maximum number within tolerance. Some tolerances are listed below. For numbers of seeds greater than those in the table and in case of additional or more extensive analyses, a tolerance based on a degree of certainty of 5 percent (P=0.05) will be recognized.

Number labeled or represented	Maximum number within tolerances	Number labeled or represented	Maximum number within tolerances
X	Y	X	Y
0	2	16	24
1	4	17	25
2	6	18	27
3	8	19	28
4	9	20	29
5	11	21	30
6	12	22	32
7	13	23	33
8	14	24	34
9	16	25	35
10	17	26	37
11	19	27	38
12	20	28	39
13	21	29	41
14	22	30	42
15	23		

[1]Quoted from Rules and Regulations of the Federal Seed Act (34). An extended tolerance table which covers labeled numbers of seeds (X) up to 300 is published in the Rules for testing seeds of the Association of Official Seed Analysts (2, pp. 91-92).

APPENDIX F: RELATIONSHIP OF CLUSTER SIZE TO TOLERANCES

Relationship of Cluster Size to Tolerances[1]

A weed or crop seed kind which is present in small amounts in a crop seed lot is said to exhibit a cluster effect when the distribution of numbers of the seeds in samples from the lot do not follow the Poisson distribution but in which numbers of clusters of the seed in samples from the lot are distributed according to the Poisson distribution.[2]

Cluster size is defined as

$$c = \frac{\text{sample variance}}{\text{sample mean}} = s^2/\bar{x} .$$

The following values of c have been determined experimentally:

Kind of seed	Substrate	c[3]
Pigweed	Red clover	5.00
Pigweed	Timothy	1.50
Canada thistle	Timothy	1.44
White clover	Timothy	1.90
White clover	Kentucky bluegrass	1.36
Alsike clover	Sweetclover	2.50

To calculate tolerances for kinds of seeds which exhibit the cluster effect, divide the labeled number of seeds by c; this gives the corresponding number of clusters per unit weight. Determine the tolerance for the number of clusters; multiply cluster tolerance by c to determine tolerance for seed.

Example: A seed lot of red clover is known to contain an average of 10 pigweed seeds per ounce. Cluster size for pigweed seeds in red clover is 5.00. To account for cluster size in application of tolerances, make calculations as follows:

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[1] In large part based on Leggatt (21, pp. 77-88), but extended by the present author.

[2] This definition (Leggatt, 1960) differs from earlier definitions of cluster size proposed by Leggatt (16, 17, 20). It is identical to the definition of h, the statistic suggested by Westmacott and Linehan (35) for use in measuring extent of homogeneity.

[3] Results of the present study indicate that c is dependent upon average number of foreign seeds per sample. Unfortunately, Leggatt did not state the average numbers of seeds per sample that were present when these values of c were determined.

Average number of seeds per ounce = 10

Average number of clusters per ounce = 10/5.00 = 2

Maximum number of clusters within tolerance (from Appendix E) = 6

Maximum number of pigweed seeds within tolerance taking cluster size into account = (6 clusters)(5 seeds/ cluster) = 30 seeds.

To account for cluster size in labeling, thus eliminating the necessity of making adjustment in application of tolerances:

Calculate maximum number of pigweed seeds within tolerance (as above) = 30 seeds

Enter tolerance table (Appendix E), column 4; find that maximum number of 30 seeds within tolerance corresponds to 21 seeds per ounce

Show on label that seeds contain an average of 21 pigweed seeds per ounce

APPENDIX G: DETAILED EXPERIMENTAL DATA

Table 15. Experiment 1. Calculation of number of red-stained alfalfa seeds in 4-, 8-, and 16-gram samples[a]

Sample size (grams)							
2		4		8		16	
Sample no.	No. of seeds	Sample no.	No. of seeds	Sample no.	No. of seeds	Sample no.	No. of seeds
1	13						
2	14	1	27				
3	12						
4	16	2	28	1	55		
5	8						
6	7	3	15				
7	11						
8	14	4	25	2	40	1	95
9	13						
10	14	5	27				
11	8						
12	14	6	22	3	49		
.							
.							
.							
.							
.							
.							
73	11						
74	9	37	20				
75	15						
76	10	38	25	19	45		
77	2						
78	9	39	11				
79	8						
80	4	40	12	20	23	10	68

[a]Treatment: mixed 2 times; substrate: unstained alfalfa seeds; replication: I. Red-stained alfalfa seeds present in 2-gram samples were observed and counted. Numbers of seeds present in 4-, 8-, and 16-gram samples were determined by addition.

Table 16. Experiment 1. Means and variances of numbers of five kinds of indicator seeds in 2-gram samples from 160-gram batches; unstained alfalfa seed substrate[a]

Batch	No. of times mixed	Replication	Red-stained alfalfa seeds		Blue-stained alfalfa seeds	
			Mean	Variance	Mean	Variance
1	2	I	9.31	9.20	8.93	9.59
2		II	9.01	12.54	9.15	13.62
3		III	9.14	12.90	9.45	10.55
4		IV	9.29	17.17	9.05	12.23
		Pooled	9.19	12.85	9.14	11.42
5	3	I	9.79	12.17	10.06	13.96
6		II	9.68	13.16	9.90	11.58
7		III	9.45	9.87	9.68	9.34
8		IV	9.59	14.14	9.81	9.70
		Pooled	9.63	12.24	9.86	11.06
9	4	I	9.23	12.61	9.09	10.33
10		II	9.28	8.83	8.86	9.31
11		III	9.16	9.25	9.11	7.54
12		IV	8.86	10.55	9.11	11.34
		Pooled	9.13	10.24	9.04	9.55
13	8	I	9.35	8.51	9.30	10.36
14		II	8.88	9.48	9.43	11.18
15		III	9.09	7.45	9.38	10.59
16		IV	9.09	7.65	9.35	10.31
		Pooled	9.10	8.22	9.36	10.51
17	16	I	9.39	8.77	9.10	8.55
18		II	9.01	9.58	9.41	10.14
19		III	9.34	12.81	9.14	10.63
20		IV	8.74	7.87	9.24	11.09
		Pooled	9.12	9.73	9.22	10.02

[a]Values from single replications are based on 80 observations; values from pooled data represent 320 observations.

Table 16 (Continued)

Batch	No. of times mixed	Replication	Curled dock seeds		Wild mustard seeds	
			Mean	Variance	Mean	Variance
1	2	I	9.25	14.47	9.38	18.49
2		II	9.73	15.44	9.93	29.31
3		III	9.70	14.79	10.01	12.44
4		IV	8.95	17.44	9.38	9.63
		Pooled	9.41	15.50	9.67	17.39
5	3	I	10.01	13.73	1.90	25.36
6		II	9.84	11.66	11.80	19.30
7		III	9.76	15.85	9.68	18.40
8		IV	9.98	12.99	9.88	14.97
		Pooled	9.89	13.44	10.81	20.41
9	4	I	9.34	16.07	9.15	8.51
10		II	10.09	12.00	9.64	24.51
11		III	9.25	10.44	9.54	11.77
12		IV	9.36	11.45	9.28	17.90
		Pooled	9.51	12.49	9.40	15.56
13	8	I	9.39	11.86	9.16	16.44
14		II	9.71	16.89	9.30	13.02
15		III	9.51	9.39	9.16	11.63
16		IV	9.21	10.75	9.19	18.81
		Pooled	9.46	12.14	9.20	14.84
17	16	I	9.41	12.27	9.16	23.38
18		II	9.93	11.59	9.79	8.88
19		III	9.23	12.78	9.59	10.32
20		IV	9.28	16.15	9.40	15.46
		Pooled	9.46	13.15	9.48	14.43

Table 16 (Continued)

Batch	No. of times mixed	Replication	Prostrate pigweed seeds	
			Mean	Variance
1	2	I	9.01	12.27
2		II	9.11	12.89
3		III	9.09	10.69
4		IV	8.95	11.04
		Pooled	9.04	11.61
5	3	I	10.21	13.94
6		II	9.88	7.82
7		III	9.36	7.27
8		IV	9.65	9.27
		Pooled	9.78	9.58
9	4	I	8.76	12.89
10		II	8.98	12.48
11		III	9.06	10.64
12		IV	8.84	10.87
		Pooled	8.91	11.63
13	8	I	9.04	8.32
14		II	9.03	9.74
15		III	9.06	13.07
16		IV	9.20	10.31
		Pooled	9.08	10.27
17	16	I	8.83	12.02
18		II	9.18	12.53
19		III	8.83	14.22
20		IV	8.95	11.04
		Pooled	8.94	12.35

Table 17. Experiment 1. Means and variances of numbers of five kinds of indicator seeds in 4-gram samples from 160-gram batches; unstained alfalfa seed substrate[a]

Batch	No. of times mixed	Replication	Red-stained alfalfa seeds		Blue-stained alfalfa seeds	
			Mean	Variance	Mean	Variance
1	2	I	18.63	23.68	17.85	21.26
2		II	18.03	34.38	18.30	31.91
3		III	18.28	38.00	18.90	18.91
4		IV	18.58	49.12	18.10	32.45
		Pooled	18.38	35.67	18.29	25.79
5	3	I	19.58	26.20	20.13	24.73
6		II	19.35	30.44	19.80	24.68
7		III	18.90	20.35	19.35	21.00
8		IV	19.18	40.51	19.63	21.93
		Pooled	19.23	28.88	19.72	22.73
9	4	I	18.45	29.23	18.18	19.64
10		II	18.55	18.25	17.72	23.03
11		III	18.33	19.15	18.23	9.15
12		IV	17.73	28.31	18.23	24.54
		Pooled	18.26	23.39	18.09	18.77
13	8	I	18.70	10.52	18.60	21.32
14		II	17.75	20.19	18.85	18.08
15		III	18.18	18.76	18.75	22.76
16		IV	18.18	16.10	18.70	25.81
		Pooled	18.20	16.20	18.73	21.58
17	16	I	18.78	15.61	18.20	14.78
18		II	18.03	14.44	18.83	22.46
19		III	18.68	26.89	18.28	22.61
20		IV	17.48	10.26	18.48	19.23
		Pooled	18.24	16.76	18.44	19.46

[a]Values from single replications are based on 40 observations; values from pooled data represent 160 observations.

Table 17 (Continued)

Batch	No. of times mixed	Replication	Curled dock seeds		Wild mustard seeds	
			Mean	Variance	Mean	Variance
1	2	I	18.50	30.41	18.75	50.76
2		II	19.45	36.92	19.85	93.00
3		III	19.40	29.11	20.03	36.28
4		IV	17.90	52.45	18.75	23.37
		Pooled	18.81	36.95	19.34	50.25
5	3	I	20.03	39.38	23.80	67.34
6		II	19.68	31.20	23.60	55.43
7		III	19.53	39.49	19.35	52.08
8		IV	19.95	39.38	19.75	43.68
		Pooled	19.78	36.45	21.62	57.96
9	4	I	18.68	37.20	18.30	19.70
10		II	20.18	33.12	19.28	84.10
11		III	18.50	26.56	19.08	25.66
12		IV	18.73	21.69	18.55	47.95
		Pooled	19.02	29.54	18.80	43.67
13	8	I	18.78	30.64	18.33	46.89
14		II	19.43	43.58	18.60	34.86
15		III	19.03	22.59	18.33	28.99
16		IV	18.43	26.87	18.38	57.88
		Pooled	13.91	30.47	18.41	41.38
17	16	I	18.83	24.25	18.33	60.74
18		II	19.85	26.03	19.58	22.66
19		III	18.45	28.25	19.18	29.53
20		IV	18.55	36.87	18.80	45.96
		Pooled	18.92	28.62	18.97	39.19

Table 17 (Continued)

Batch	No. of times mixed	Replication	Prostrate pigweed seeds	
			Mean	Variance
1	2	I	18.03	29.61
2		II	18.23	35.31
3		III	18.18	24.66
4		IV	17.90	25.99
		Pooled	18.08	28.36
5	3	I	20.43	31.99
6		II	19.78	13.56
7		III	18.73	16.56
8		IV	19.30	17.86
		Pooled	19.56	20.01
9	4	I	17.53	31.44
10		II	17.95	22.46
11		III	18.13	22.83
12		IV	17.68	20.43
		Pooled	17.82	23.89
13	8	I	18.08	22.58
14		II	18.05	19.54
15		III	18.13	27.24
16		IV	18.40	25.02
		Pooled	18.16	23.17
17	16	I	17.65	21.52
18		II	18.35	27.46
19		III	17.65	35.21
20		IV	17.90	26.09
		Pooled	17.89	27.13

Table 18. Experiment 1. Means and variances of numbers of five kinds of indicator seeds in 8-gram samples from 160-gram batches; unstained alfalfa seed substrate[a]

Batch	No. of times mixed	Replication	Red-stained alfalfa seeds		Blue-stained alfalfa seeds	
			Mean	Variance	Mean	Variance
1	2	I	37.25	65.67	35.70	40.01
2		II	36.05	97.94	36.60	109.20
3		III	36.55	110.05	37.80	42.69
4		IV	37.15	149.29	36.20	94.69
		Pooled	36.75	101.96	36.58	69.54
5	3	I	39.15	74.34	40.25	57.25
6		II	38.70	49.48	39.60	54.88
7		III	37.80	60.27	38.70	55.27
8		IV	38.35	108.66	39.25	48.09
		Pooled	38.50	70.66	39.45	52.15
9	4	I	36.90	77.57	36.35	46.03
10		II	37.10	35.36	35.45	52.26
11		III	36.65	44.98	36.45	14.36
12		IV	35.45	71.42	36.45	53.73
		Pooled	36.53	55.57	36.18	40.20
13	8	I	37.40	28.57	37.20	35.64
14		II	35.50	43.00	37.70	36.33
15		III	36.35	43.08	37.50	55.95
16		IV	36.35	34.87	37.40	45.73
		Pooled	36.40	36.42	37.45	41.80
17	16	I	37.55	25.94	36.40	30.46
18		II	36.05	36.37	37.65	32.98
19		III	37.35	58.56	36.55	18.89
20		IV	34.95	21.10	36.95	50.79
		Pooled	36.48	35.27	36.89	32.25

[a]Values from single replications are based on 20 observations; values from pooled data represent 80 observations.

Table 18 (Continued)

Batch	No. of times mixed	Replication	Curled dock seeds		Wild mustard seeds	
			Mean	Variance	Mean	Variance
1	2	I	37.00	87.68	37.50	165.21
2		II	38.90	105.67	39.70	240.43
3		III	38.80	73.85	40.04	77.84
4		IV	35.80	181.96	37.50	68.26
		Pooled	37.63	109.73	38.69	134.14
5	3	I	40.05	93.63	47.60	172.67
6		II	39.35	96.24	47.20	171.43
7		III	39.05	124.68	38.70	163.06
8		IV	39.90	98.09	39.50	127.84
		Pooled	39.59	99.41	43.25	170.27
9	4	I	37.35	83.82	36.60	57.20
10		II	40.35	82.98	38.55	301.21
11		III	37.00	76.21	38.15	65.08
12		IV	37.45	48.37	37.10	135.67
		Pooled	38.04	71.91	37.60	135.10
13	8	I	37.55	86.05	36.65	151.92
14		II	38.85	139.82	37.20	100.91
15		III	38.05	57.10	36.65	88.24
16		IV	36.85	70.98	36.75	151.67
		Pooled	37.83	85.67	36.81	118.56
17	16	I	37.65	56.87	36.65	224.03
18		II	39.70	47.27	39.15	59.61
19		III	36.90	75.04	38.35	81.92
20		IV	37.10	113.88	37.60	122.57
		Pooled	37.84	71.73	37.94	118.26

Table 18 (Continued)

Batch	No. of times mixed	Replication	Prostrate pigweed seeds	
			Mean	Variance
1	2	I	36.05	89.00
2		II	36.45	89.42
3		III	36.35	64.98
4		IV	35.80	73.22
		Pooled	36.16	76.21
5	3	I	40.85	72.24
6		II	39.55	32.26
7		III	37.45	31.10
8		IV	38.60	43.20
		Pooled	39.11	44.58
9	4	I	35.05	100.58
10		II	35.90	61.57
11		III	36.25	53.25
12		IV	35.35	53.29
		Pooled	35.64	64.84
13	8	I	36.15	64.98
14		II	36.10	42.83
15		III	36.25	87.78
16		IV	36.80	69.75
		Pooled	36.33	63.89
17	16	I	35.30	49.38
18		II	36.70	74.54
19		III	35.30	85.48
20		IV	35.80	86.17
		Pooled	35.78	71.42

Table 19. Experiment 1. Means and variances of numbers of five kinds of indicator seeds in 16-gram samples from 160-gram batches; unstained alfalfa seed substrate[a]

Batch	No. of times mixed	Replication	Red-stained alfalfa seeds		Blue-stained alfalfa seeds	
			Mean	Variance	Mean	Variance
1	2	I	74.50	170.50	71.40	91.60
2		II	72.10	368.10	73.20	380.84
3		III	73.10	415.21	75.60	96.04
4		IV	74.30	479.34	72.40	277.60
		Pooled	73.50	331.69	73.15	197.72
5	3	I	78.30	204.90	80.50	150.28
6		II	77.40	119.16	79.20	127.07
7		III	75.60	185.38	77.40	90.93
8		IV	76.70	237.12	78.50	146.94
		Pooled	77.00	173.28	78.90	120.19
9	4	I	73.80	258.40	72.70	115.57
10		II	74.20	82.40	70.90	130.54
11		III	73.30	44.23	72.90	37.21
12		IV	70.90	83.43	72.90	78.99
		Pooled	73.05	109.79	72.35	84.34
13	8	I	74.80	54.40	74.40	51.82
14		II	71.00	121.11	75.40	54.27
15		III	72.70	106.90	75.00	150.00
16		IV	72.70	52.01	74.80	68.18
		Pooled	72.80	79.04	74.90	74.96
17	16	I	75.10	57.66	72.80	77.73
18		II	72.10	55.88	75.30	60.68
19		III	74.70	91.12	73.10	27.88
20		IV	69.90	46.10	73.90	91.43
		Pooled	72.95	62.41	73.78	60.44

[a]Values from single replications are based on 10 observations; values from pooled data represent 40 observations.

Table 19 (Continued)

Batch	No. of times mixed	Replication	Curled dock seeds		Wild mustard seeds	
			Mean	Variance	Mean	Variance
1	2	I	74.00	248.67	75.00	636.67
2		II	77.80	276.40	79.40	875.42
3		III	77.60	175.16	80.10	258.32
4		IV	71.60	642.04	75.00	195.56
		Pooled	75.25	316.65	77.38	459.63
5	3	I	80.10	243.88	95.20	656.84
6		II	78.70	297.57	94.40	611.16
7		III	78.10	418.10	77.40	375.60
8		IV	79.80	269.07	79.00	485.78
		Pooled	79.18	284.20	86.50	562.46
9	4	I	74.70	287.79	73.20	109.73
10		II	80.70	256.01	77.10	856.10
11		III	74.00	250.89	76.30	226.46
12		IV	74.90	140.10	74.20	448.18
		Pooled	76.08	223.15	75.20	381.09
13	8	I	75.10	316.10	73.30	522.68
14		II	77.70	462.01	74.40	302.71
15		III	76.10	207.66	73.30	230.01
16		IV	73.70	108.68	73.50	412.06
		Pooled	75.65	229.11	73.63	338.86
17	16	I	75.30	106.68	73.30	878.46
18		II	79.40	132.27	78.30	150.23
19		III	73.80	233.73	76.70	249.12
20		IV	74.20	321.96	75.20	400.18
		Pooled	75.68	188.43	75.88	390.73

Table 19 (Continued)

Batch	No. of times mixed	Replication	Prostrate Pigweed seeds	
			Mean	Variance
1	2	I	72.10	317.66
2		II	72.90	268.99
3		III	72.70	89.79
4		IV	71.60	200.93
		Pooled	72.33	202.74
5	3	I	81.70	69.34
6		II	79.10	104.54
7		III	74.90	54.10
8		IV	77.20	135.07
		Pooled	78.23	90.18
9	4	I	70.10	274.77
10		II	71.80	73.96
11		III	72.50	198.28
12		IV	70.70	156.90
		Pooled	71.28	163.33
13	8	I	72.30	168.68
14		II	72.20	128.40
15		III	72.50	305.83
16		IV	73.60	177.38
		Pooled	72.65	180.39
17	16	I	70.60	91.60
18		II	73.40	141.82
19		III	70.60	213.60
20		IV	71.60	254.49
		Pooled	71.55	163.23

Table 20. Experiment 1. Comparison of homogeneity tests. Red-stained alfalfa seeds in unstained alfalfa seed substrate. Data presented in terms of hererogeneity declarations (4 possible)

Sample size (grams)	No. of times mixed	Test made			
		Legatt	H[a]	Long	Short
2	2	3	0	0	Tests not made[b]
	3	2	0	0	
	4	1	0	0	
	8	0	0	0	
	16	1	0	0	
	Subtotal (20 possible)	7	0	0	
4	2	3	2	1	Tests not made[b]
	3	2	1	0	
	4	2	0	0	
	8	0	0	0	
	16	1	0	0	
	Subtotal (20 possible)	8	3	1	
8	2	4	3	3	2
	3	3	1	1	1
	4	2	2	0	0
	8	0	0	0	0
	16	0	0	0	0
	Subtotal (20 possible)	9	6	4	3
16	2	4	4	3	3
	3	3	3	0	1
	4	1	1	1	1
	8	0	0	0	0
	16	0	0	0	0
	Subtotal (20 possible)	8	8	4	5
TOTAL	(80 possible)	32	17	9	-

[a]Critical value of 1.00.

[b]Limits for tests involving over 20 observations were not given by the authors (25).

Table 21. Experiment 1. Comparison of homogeneity tests. Blue-stained alfalfa seeds in unstained alfalfa seed substrate. Data presented in terms of heterogeneity declarations (4 possible)

Sample size (grams)	No. of times mixed	Test made			
		Leggatt	H[a]	Long	Short
2	2	2	0	0	Tests not made[b]
	3	1	0	0	
	4	0	0	0	
	8	0	0	0	
	16	0	0	0	
	Subtotal (20 possible)	3	0	0	
4	2	2	0	0	Tests not made[b]
	3	0	0	0	
	4	0	0	0	
	8	0	0	0	
	16	0	0	0	
	Subtotal (20 possible)	2	0	0	
8	2	2	2	2	0
	3	0	0	0	0
	4	0	0	0	0
	8	0	0	0	0
	16	0	0	0	0
	Subtotal (20 possible)	2	2	2	0
16	2	2	2	2	1
	3	0	0	0	0
	4	0	0	0	0
	8	1	1	0	0
	16	0	0	0	0
	Subtotal (20 possible)	3	3	2	1
TOTAL	(80 possible)	10	5	4	-

[a]Critical value of 1.00.

[b]Limits for tests involving over 20 observations were not given by the authors (25).

Table 22. Experiment 1. Comparison of homogeneity tests. Curled dock seeds in unstained alfalfa seed substrate. Data presented in terms of heterogeneity declarations (4 possible)

Sample size (grams)	No. of times mixed	Test made			
		Leggatt	H[a]	Long	Short
2	2	4	0	0	Tests not made[b]
	3	3	0	0	
	4	1	0	0	
	8	1	0	0	
	16	3	0	0	
	Subtotal (20 possible)	12	0	0	
4	2	4	1	1	Test not made[b]
	3	4	1	0	
	4	3	0	0	
	8	3	1	0	
	16	2	0	0	
	Subtotal (20 possible)	16	3	1	
8	2	4	3	2	2
	3	4	4	1	3
	4	3	3	0	0
	8	3	2	1	1
	16	2	2	1	1
	Subtotal (20 possible)	16	14	5	7
16	2	4	4	3	3
	3	4	4	3	4
	4	3	3	2	3
	8	3	3	2	2
	16	2	2	1	2
	Subtotal (20 possible)	16	16	11	14
TOTAL (80 possible)		60	33	17	— -

[a]Critical value of 1.00.

[b]Limits for tests involving over 20 observations were not given by the authors (25).

Table 23. Experiment 1. Comparison of homogeneity tests. Wild mustard seeds in unstained alfalfa seed substrate. Data presented in terms of heterogeneity declarations (4 possible)

Sample size (grams)	No. of times mixed	Test made			
		Leggatt	H[a]	Long	Short
2	2	2	1	1	Tests not made[b]
	3	4	1	1	
	4	2	1	1	
	8	3	1	0	
	16	2	1	1	
	Subtotal (20 possible)	13	5	4	
4	2	3	2	2	Tests not made[b]
	3	4	4	3	
	4	2	2	2	
	8	4	2	2	
	16	3	2	0	
	Subtotal (20 possible)	16	12	9	
8	2	4	2	2	2
	3	4	4	4	4
	4	3	2	2	2
	8	4	4	3	4
	16	3	3	2	2
	Subtotal (20 possible)	18	15	13	14
16	2	3	3	2	2
	3	1	1	1	0
	4	3	3	1	1
	8	3	3	1	1
	16	3	2	1	1
	Subtotal (20 possible)	13	12	6	5
TOTAL (80 possible)		60	44	32	-

[a]Critical value of 1.00.

[b]Limits for tests involving over 20 observations were not given by the authors (25).

Table 24. Experiment 1. Comparison of homogeneity tests. Prostrate pigweed seeds in unstained alfalfa seed substrate. Data presented in terms of heterogeneity declarations (4 possible)

Sample size (grams)	No. of times mixed	Test made			
		Leggatt	H[a]	Long	Short
2	2	2	0	0	Tests
	3	1	0	0	not
	4	2	0	0	made[b]
	8	1	0	0	
	16	3	0	0	
	Subtotal (20 possible)	9	0	0	
4	2	3	0	0	Tests
	3	1	0	0	not
	4	1	0	0	made[b]
	8	1	0	0	
	16	3	0	0	
	Subtotal (20 possible)	9	0	0	
8	2	4	3	0	0
	3	1	0	0	0
	4	2	1	1	1
	8	3	1	0	1
	16	3	3	0	1
	Subtotal (20 possible)	13	8	1	3
16	2	3	3	2	2
	3	1	1	1	0
	4	3	3	1	1
	8	3	3	1	1
	16	3	2	1	1
	Subtotal (20 possible)	13	12	6	5
TOTAL (80 possible)		44	20	7	-

[a]Critical value of 1.00.

[b]Limits for tests involving over 20 observations were not given by the authors (25).

Table 25. Experiment 1. Percentage of samples which contained indicator seeds in numbers which exceed tolerance limits of the Federal Seed Act (Appendix E)[a]

Sample size (grams)	No. of times mixed	Red-stained alfalfa	Blue-stained alfalfa	Curled dock	Wild mustard	Prostrate pigweed
		%	%	%	%	%
2	2	1.3	1.6	2.8	3.8	1.6
	3	1.3	0.9	2.2	5.6	1.3
	4	0.3	0.9	1.9	3.1	1.9
	8	0.0	0.3	1.9	2.5	0.6
	16	0.3	0.9	2.2	2.8	1.6
4	2	5.6	5.0	7.5	5.6	4.4
	3	2.5	3.8	8.8	10.6	3.1
	4	1.9	1.3	5.0	4.4	3.1
	8	0.6	1.9	5.6	5.6	2.5
	16	0.0	0.6	3.8	4.4	4.4
8	2	8.8	5.0	15.0	10.0	10.0
	3	8.8	8.8	13.8	20.0	2.5
	4	3.8	3.8	8.8	7.5	2.5
	8	1.3	2.5	11.3	7.5	5.0
	16	1.3	0.0	8.8	10.0	6.3
16	2	15.0	7.5	17.5	17.5	7.5
	3	15.0	10.0	17.5	32.5	10.0
	4	5.0	2.4	12.5	10.0	7.5
	8	5.0	2.5	15.0	12.5	5.0
	16	0.0	2.5	12.5	10.0	7.5

[a]Each entry in table was calculated from pooled data of 4 replications. Numbers of observations represented in each entry were as follows:

2-gram samples: 320 observations
4-gram samples: 160 observations
8-gram samples: 80 observations
16-gram samples: 40 observations.

Table 26. Experiment 3. Comparison of homogeneity tests. Blue-stained alfalfa seeds in unstained alfalfa seed substrate; batches mixed 2 times

Sample size (grams)	Test applied	No. of heterogeneity declarations (10 possible) Samples per group 5	10	20	Total (30 possible)
2	Leggatt	0	0	2	2
	H	2	0	1	3
	Long	0	0	0	0
	Short	0	0	0	0
4	Leggatt	3	3	4	10
	H	3	2	1	6
	Long	0	0	0	0
	Short	0	0	0	0
8	Leggatt	2	6	8	16
	H	4	6	4	14
	Long	0	0	1	1
	Short	0	0	0	0
16	Leggatt	2	8	10	20
	H	4	8	9	21
	Long	0	3	5	8
	Short	0	3	0	3

Table 27. Experiment 3. Comparison of homogeneity tests. Blue-stained alfalfa seeds in unstained alfalfa seed substrate; batches mixed 4 times

Sample size (grams)	Test applied	No. of heterogeneity declarations (10 possible) Samples per group			Total (30 possible)
		5	10	20	
2	Leggatt	1	2	2	5
	H	2	2	1	5
	Long	0	0	0	0
	Short	0	0	0	0
4	Leggatt	1	3	0	4
	H	1	3	0	4
	Long	0	1	0	1
	Short	0	1	0	1
8	Leggatt	1	3	0	4
	H	2	3	0	5
	Long	0	0	0	0
	Short	0	0	0	0
16	Leggatt	1	3	0	4
	H	1	1	0	2
	Long	0	0	0	0
	Short	0	0	0	0

Table 28. Experiment 3. Comparison of homogeneity tests. Blue-stained alfalfa seeds in unstained alfalfa seed substrate; batches mixed 16 times

Sample size (grams)	Test applied	No. of heterogeneity declarations (10 possible) Samples per group 5	10	20	Total (30 possible)
2	Leggatt	1	0	1	2
	H	1	0	0	1
	Long	0	0	0	0
	Short	0	0	0	0
4	Leggatt	1	0	0	1
	H	1	0	0	1
	Long	0	0	0	0
	Short	0	0	0	0
8	Leggatt	1	0	0	1
	H	1	0	0	1
	Long	0	0	0	0
	Short	0	0	0	0
16	Leggatt	1	0	0	1
	H	1	0	0	1
	Long	0	0	0	0
	Short	0	0	0	0

Table 29. Experiment 4. Variance of numbers of stained rape seeds present in samples of different sizes from 320-gram batches; unstained rape seed substrate; five indicator seed concentrations; six mixing treatments

No. of times mixed	Replication	Mean no. indicator seeds per sample				
		5	10			
		Sample size (grams)				
		16	4	8	16	32
1	I	6.42			27.17	15.78
	II	8.00			30.83	17.33
	III	8.20			20.89	23.51
	IV	7.21			27.31	19.21
	Pooled	7.18			24.21	17.51
2	I	8.79	11.11	11.25	11.73	11.88
	II	5.00	12.13	11.97	10.79	12.12
	III	8.47	12.28	11.07	12.83	9.21
	IV	6.00	15.32	9.49	16.89	15.66
	Pooled	6.80	12.60	10.76	12.57	11.35
3	I	5.63			7.63	11.66
	II	4.74			7.73	11.78
	III	3.16			11.16	6.00
	IV	4.84			11.58	7.33
	Pooled	4.42			9.16	8.49
4	I	4.53			10.05	9.78
	II	2.26			9.52	4.54
	III	3.63			8.21	4.54
	IV	4.63			17.26	14.67
	Pooled	3.62			10.83	7.74
8	I	6.32			11.84	9.78
	II	3.88			10.09	9.51
	III	4.53			9.68	9.11
	IV	3.63			10.00	4.32
	Pooled	4.42			10.04	7.56
16	I	4.21			7.15	8.00
	II	5.37			9.67	18.22
	III	4.32			7.58	5.11
	IV	6.32			7.73	10.67
	Pooled	4.86			7.73	9.69

Table 29 (Continued)

No. of times mixed	Replication	Mean no. indicator seeds per sample 20 Sample size (grams) 8	16	32
1	I		90.99	58.04
	II		39.78	73.29
	III		141.42	81.88
	IV		77.08	91.88
	Pooled		84.03	70.83
2	I	21.33	29.06	34.77
	II	26.87	26.83	18.98
	III	32,88	21.84	29.29
	IV	32.76	36.16	53.66
	Pooled	27.95	27.42	31.56
3	I		17.12	13.43
	II		38.74	14.99
	III		19.79	25.78
	IV		21.52	28.89
	Pooled		23.38	19.18
4	I		17.21	10.10
	II		16.21	28.99
	III		14.94	15.11
	IV		28.95	36.89
	Pooled		18.59	21.02
8	I		39.27	30.32
	II		27.25	18.18
	III		14.79	10.22
	IV		12.73	14.22
	Pooled		22.97	16.97
16	I		23.42	22.84
	II		39.06	24.18
	III		18.47	18.00
	IV		17.99	20.10
	Pooled		23.82	19.67

Table 29 (Continued)

No. of times mixed	Replication	Mean no. indicator seeds per sample		
		40		80
		Sample size (grams)		
		16	32	32
1	I	257.50	314.40	1004.46
	II	227.04	99.29	799.17
	III	285.64	551.43	941.82
	IV	135.29	230.01	279.12
	Pooled	217.77	275.91	697.98
2	I	58.79	71.60	134.32
	II	117.31	80.62	427.88
	III	66.77	43.43	215.57
	IV	75.47	118.10	284.00
	Pooled	76.72	72.50	245.66
3	I	47.84	40.27	73.82
	II	44.22	105.11	249.79
	III	72.93	20.22	75.73
	IV	21.62	51.66	91.83
	Pooled	42.05	50.16	104.13
4	I	33.57	48.77	48.72
	II	53.73	33.56	84.54
	III	21.14	27.07	14.94
	IV	49.88	74.44	129.83
	Pooled	38.09	42.44	64.19
8	I	41.78	36.49	93.96
	II	81.08	65.38	208.90
	III	37.53	80.18	45.11
	IV	29.88	6.54	75.29
	Pooled	45.83	30.78	97.94
16	I	31.25	64.54	53.07
	II	39.00	79.82	82.54
	III	35.46	44.99	70.50
	IV	46.32	30.28	66.22
	Pooled	36.61	50.77	63.04

Table 30. Experiment 4. Comparison of homogeneity tests. Yellow-stained rape seeds in unstained rape seed substrate. Data presented in terms of heterogeneity declarations (4 possible)

Sample size (grams)	No. of times mixed	Test applied	Mean no. indicator seeds per sample					Total (16 possible)
			5	10	20	40	80	
4	2	Leggatt		0				
		H		0				
		Long		0				
8	2	Leggatt		0	2			
		H		0	0			
		Long		0	0			
16	1	Leggatt	0	4	4	4		12
		H	0	4	3	4		11
		Long	0	0	3	4		7
		Short	0	1	4	4		9
	2	Leggatt	0	0	1	4		5
		H	0	0	0	1		1
		Long	0	0	0	1		1
		Short	0	0	0	1		1
	3	Leggatt	0	0	1	1		2
		H	0	0	0	0		0
		Long	0	0	0	0		0
		Short	0	0	0	0		0
	4	Leggatt	0	0	0	0		0
		H	0	0	0	0		0
		Long	0	0	0	0		0
		Short	0	0	0	0		0
	8	Leggatt	0	0	1	1		2
		H	0	0	0	1		1
		Long	0	0	0	0		0
		Short	0	0	0	0		0
	16	Leggatt	0	0	1	0		1
		H	0	0	0	0		0
		Long	0	0	0	0		0
		Short	0	0	0	0		0

Table 30 (Continued)

Sample size (grams)	No. of times mixed	Test applied	Mean no. indicator seeds per sample				Total (16 possible)
			5	10	20	40	80
32	1	Leggatt		2	4	4	4	14
		H		1	4	4	4	13
		Long		0	4	4	4	12
		Short		0	4	3	3	10
	2	Leggatt		0	2	3	4	9
		H		0	1	2	3	6
		Long		0	1	1	3	5
		Short		0	0	1	1	2
	3	Leggatt		0	0	1	1	2
		H		0	0	1	1	2
		Long		0	0	1	1	2
		Short		0	0	0	1	1
	4	Leggatt		0	1	1	1	3
		H		0	0	0	0	0
		Long		0	0	0	0	0
		Short		0	0	0	0	0
	8	Leggatt		0	0	2	1	3
		H		0	0	1	1	2
		Long		0	0	0	1	1
		Short		0	0	0	0	0
	16	Leggatt		1	0	2	0	3
		H		0	0	0	0	0
		Long		0	0	0	0	0
		Short		0	0	0	0	0

Table 31. Experiment 4. Percentage of samples which contained indicator seeds[a] in numbers which exceed tolerance limits of the Federal Seed Act

Mean No. of indicator seeds per sample	No. of times mixed	Sample size (grams)			
		4	8	16	32
		%	%	%	%
5	1			1.3	
	2			2.5	
	3			0.0	
	4			0.0	
	8			0.0	
	16			0.0	
10	1			5.0	0.0
	2	2.2	3.1	1.3	0.0
	3			1.3	0.0
	4			2.5	0.0
	8			1.3	0.0
	16			1.3	0.0
20	1			8.8	7.5
	2		1.9	3.8	5.0
	3			3.8	0.0
	4			1.3	2.5
	8			3.0	0.0
	16			2.5	0.0
40	1			15.0	17.5
	2			3.8	8.8
	3			4.2	2.5
	4			3.8	0.0
	8			1.3	0.0
	16			0.0	5.0
80	1				25.0
	2				12.5
	3				5.0
	4				2.5
	8				0.0
	16				0.0

[a]Stained rape seeds in an unstained rape seed substrate.